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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

Certificate Under 37 CFR 1.10
Date of Deposit: June 25, 2004
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PO Box 1450
Alexandria, VA 22313-1450
(703) 308-4357

This is a request for filing a Provisional Application for Patent under 37 CFR 1.53(c)

Inventor(s) and Residence(s) (city and either state or foreign country):

Last Name	First Name	Middle Initial	City	State or Country
Rekosh	David		Earlysville	Virginia
Hammarskjöld	Marie-Louise		Earlysville	Virginia

Title: **COMPOUNDS THAT INHIBIT HIV PARTICLE FORMATION FOR THE TREATMENT OF AIDS**

112 Sheets of specification.
7 Sheets of drawings.

University of Virginia Patent Foundation claims small entity status as a nonprofit organization (37 CFR §§1.27(a)(3) and (c)). The Commissioner is hereby authorized to charge the Small Entity Fee of **$80** to **Deposit Account No. 50-0423.**

Please direct all communication relating to this application to:
John P. Breen, Esq.
Patent Counsel
University of Virginia Patent Foundation
1224 West Main Street, Suite 1-110
Charlottesville, VA 22903 U.S.A.
Customer No. 34444
Telephone: (434) 243-6103
Fax: (434) 924-2493

This invention was made by an agency of the United States Government or under a contract with an agency of the United States Government. The government has certain rights in the invention.

YES ☒ NO ☐ Grant No. R21 AI54213-01 and R21 AI54213-02

Dated: June 25, 2004

Respectfully submitted,

By: _____
John P. Breen (Reg. No. 38,833)

Compounds that inhibit HIV particle formation for the treatment of AIDS

US Government Rights

This invention was made with United States Government support under Grant Nos. R21 AI54213-01, and R21 AI54213-02, awarded by National Institutes of Health. The United States Government has certain rights in the invention.

Background

The present invention is directed to a safe quantitative *in vitro* high-throughput assay to screen library compounds for effects on Rev-dependent p24 production. Two cell lines derived from COS cells provide the means of determining whether a library compound has anti-Rev activity; 5BD.1 and 2A.22. These cell lines constitutively express HIV-like particles that contain the HIV core proteins as well as HIV envelope protein. The non-infectious virions created by these cells are secreted into the media, where a simple p24 ELISA can quantitatively determine virion production.

The Production of these virus-like particles is totally dependent on the expression of the Rev protein (for the 5BD.1 cell line), which is also made in these cell lines, or independent of Rev protein expression (the 2A.22 cell line). The cell lines are useful as packaging cells for HIV vectors, and also for drug screening using the HIV Rev protein as a target. The 5BD.1 and 5BA.1 cell lines described here are a considerable improvement over the previous B4.14 cell line, in that they express the HIV envelope protein, which the B4.14 cell lines does not and the cell also produces higher levels of p24.

The HIV virus particle consists of internal proteins that make up the viral core and two proteins that are part of the lipid envelope that surrounds the core. These proteins are expressed from precursor molecules called Pr55gag and Pr160gagpol for the core proteins and gp160 for the envelope proteins. Studies in our laboratory and elsewhere have demonstrated expression of these proteins normally requires co-expression of the HIV Rev protein. Without the Rev protein, the mRNAs encoding each of these proteins remains in the nucleus. In order for the Rev protein to work, it is also essential to have an element present in the RNA that binds to Rev. This element is called the RRE.

Using a vector that expressed Pr55gag and Pr160 and a vector that expressed Rev, we originally created the cell line called B4.14. This cell line was made at the State University of New York in Buffalo. B4.14 expressed HIV-like particles without the envelope protein. The creation of this cell line was described in the following publication: Srinivasakumar, N., Chazal, N., Helga-Maria,C., Prasad, S., Hammmarskjöld, M.-L., and Rekosh, D. (1997) The effect of viral regulatory protein expression on gene delivery by human immunodeficiency virus type 1 vectors produced in stable packaging cell lines. J. Virol 71:5841-5848 .

At the University of Virginia, the B4.14 cell line was modified to also express the HIV envelope protein, by transfecting the cell line with a vector that expressed the protein. The resulting cell lines isolated are called 5BD.1 and 5BA.1. 5BA.1 is simply a different clone that was isolated at the same time as 5BD.1. There appears to be little difference in the properties of the two cell lines. One feature of these cell lines, relative to the parental B4.14, is that they express 2-4 times more p24 (HIV core) protein depending on the culture conditions.

Work in our laboratory led to the identification of a small RNA element from Mason-Pfizer Monkey Virus. When this element is present in the RNA that is expressed from a gene that normally requires Rev co-expression the need for Rev is overcome.

Our initial findings are described in detail in two patents and published paper:

"Purified retroviral constitutive transport enhancer elements that enhance nucleo-cytoplasmic transport of mRNA and methods of making and using the elements" US Patent # 5880276 Issued 3/9/99.

"A purified retroviral constitutive transport enhancer and its use to facilitate mRNA transport, and to produce recombinant, attenuated HIV" US Patent # 5585263 Issued 12/17/96.

Bray, M., Prasad, S. Dubay, J.W., Hunter, E. Jeang, K.T., Rekosh, D. and Hammarskjöld-M-L. (1994) A small element from the Mason-Pfizer monkey virus genome makes human immunodeficiency virus type 1 expression and replication Rev-independent. Proc. Natl. Acad.Sci.(USA) 91: 1256-1260.

Using the CTE as a component of our expression vectors has allowed us to, create a series of expression vectors that allows expression of HIV proteins in a Rev-independent fashion. The vectors were then used to create stable cell lines that expressed the proteins. One cell line in particular has proved extremely useful. It is called 2A.22. The cell line expressed HIV proteins (Gag-GagPol and Envelope) in a Rev-independent fashion. The creation of this cell line and its properties was described in the following publication: Srinivasakumar, N., Chazal, N., Helga-Maria,C., Prasad, S., Hammmarskjöld, M.-L., and Rekosh, D. (1997) The effect of viral regulatory protein expression on gene delivery by human immunodeficiency virus type 1 vectors produced in stable packaging cell lines. J. Virol 71:5841-5848.

We are currently modifying 2A.22 by transfection with a vector that produces secreted alkaline phosphatase (SEAP). Since the SEAP to p24 ratio can readily be measured simply by assaying the medium. having such a modification will allow us, in a drug screening assay, to readily identify compounds that inhibit p24 production, but not the control SEAP gene.

One of the two necessary regulatory genes in the HIV genome, Rev initiates export of full length and partially processed HIV RNAs from the nucleus to the cytoplasm, a necessary event for HIV replication. Rev functions through the binding of RNA encoded Rev responsive element (RRE), an approximately 230 nucleotide sequence, followed by binding to cellular CRM1. This ribonuclear protein complex is then shuttled out of the nucleus using other cellular machinery in the nuclear pore.

RNA export elements are found in other RNA viruses. One example is the Mason-Pfizer monkey virus (MPMV) constitutive transport element (CTE). Similar to the RRE, the CTE is a small nucleotide sequence found on full length and partially processed MPMV RNAs. Circumventing the need for an MPMV analog of Rev, the CTE attaches directly to cellular machinery to initiate nuclear export. Experiments have shown that replacing the HIV RRE with a MPMV CTE leads to Rev-independent HIV-1 RNA export to the cytoplasm.

The cell line 5BD.1 was created by transfecting COS cells with the wild type HIV-1 structural and regulatory genes *gag*, *gagpol*, *rev*, and *env*. Each of these genes are necessary but not sufficient for producing infectious HIV virions. Non-infectious virions are produced

in 5BD.1 cells via the same pathways as in $CD4^+$ cells. Inhibition of Rev with a library compound would therefore have the same effect on viral production in 5BD.1 cells as in $CD4^+$ cells.

2A.22 was created by transfecting COS cells with modified HIV-1 structural genes gag, gagpol, and env. These genes were modified to replace the Rev binding site with the MPMV CTE. This cell line produces non-infectious virions in a Rev-independent manner. The Rev-independence of 2A.22 is useful as a negative control while testing library compounds. When both cell lines 5BD.1 and 2A.22 are grown and tested with the same compound under similar conditions, a reduction in viral production in 5BD.1 and not in 2A.22 indicates a potentially positive score for that compound as a Rev-specific inhibitor. Alternately, if a compound reduced p24 levels in both 5BD.1 and 2A.22, this could indicate a possible harmful interaction with cellular machinery and would rule out that specific compound from further studies.

There is clear evidence that Human Immunodeficiency Virus (HIV) is the cause of AIDS and that drugs that inhibit the replication and production of infectious HIV particles are efficacious in the treatment of AIDS. This disclosure describes 12 compounds that we have discovered that are very effective inhibitors of HIV particle formation. The compounds may act by inhibiting HIV Rev function, HIV assembly, HIV particle budding or some other part of the HIV life cycle. The compounds are therefore likely to form the chemical basis for new drugs that could be used for the treatment of AIDS.

The inhibitory compounds of the present invention were identified using the 5BD.1 cell line to screen for drugs that inhibit HIV particle formation without showing toxicity in a 5 day cell survival assay. The amount of HIV particles released by budding from the 5BD.1 cell line into the culture medium was measured using a simple and straightforward ELISA assay. 40,000 compounds were screened and 12 were selected as "hits" based on their ability to inhibit HIV particle formation without showing toxicity in a 5 day cell survival assay.

Brief Summary

The present invention is directed to novel HIV inhibitory compounds and the use of those compounds to treat patients that are HIV positive.

Brief Summary of the Drawings

Fig. 1 parts A- I show the ELISA data readout from the primary screen of 40,000 compounds plotted as a percentage of the control. Compounds that gave an inhibition of HIV particle formation below 50% were chosen for further study. There are 192 compounds that give values below 50%.

Fig. 2 shows the chemical structures and names of the 12 compounds being disclosed.

Fig. 3 shows a three concentration dose response experiment for the compounds added to the cell line. Each compound is named with an identifier along the X-axis of the graph. The Y-axis shows the percent inhibition of HIV particle release into the medium. The different concentrations of compound utilized are represented by the different color bars as shown in the figure legend. Details of the assay are given in part E.

Fig. 4 shows a six concentration dose response curve of the same compounds. Details are the same as for Fig 3 except that six concentrations of compound were tested.

Figs. 5 and 6 show 2 day MTS toxicity assays for the compounds in 5 BD.1 cells, the same cell line used for the drug screening. A three concentration MTS toxicity assay is shown in Fig 5 and a six concentration assay is shown in Fig 6. The Y-axis represents the percentage of live cells after two days of treatment with the compounds. The same concentrations used in Fig 3 and 4 are used in this figure, as indicated by the different colors. Details of the assay are given in part E.

Fig. 7 shows a six concentration cell viability assay using the MT-4 T-cell line. The Y-axis shows the number of cells surviving after 5 days of incubation with each compound. The concentration of each compound used is indicated by the different colors. The data is expressed as the percentage of the starting number of cells. Details of this assay are given in part E.

Detailed Description of Embodiments

Definitions

In describing and claiming the invention, the following terminology will be used in accordance with the definitions set forth below.

As used herein, the term "purified" and like terms relate to an enrichment of a molecule or compound relative to other components normally associated with the molecule or compound in a native environment. The term "purified" does not necessarily indicate that complete purity of the particular molecule has been achieved during the process. A "highly purified" compound as used herein refers to a compound that is greater than 90% pure.

As used herein, the term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents. The term also encompasses any of the agents approved by a regulatory agency of the US Federal government or listed in the US Pharmacopeia for use in animals, including humans.

As used herein, the term "treating" includes prophylaxis of the specific disorder or condition, or alleviation of the symptoms associated with a specific disorder or condition and/or preventing or eliminating said symptoms.

As used herein, the term "halogen" or "halo" includes bromo, chloro, fluoro, and iodo.

The term "haloalkyl" as used herein refers to an alkyl radical bearing at least one halogen substituent, for example, chloromethyl, fluoroethyl or trifluoromethyl and the like.

The term "C_1-C_n alkyl" wherein n is an integer, as used herein, represents a branched or linear alkyl group having from one to the specified number of carbon atoms. Typically C_1-C_6 alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, butyl, iso-butyl, sec-butyl, tert-butyl, pentyl, hexyl and the like.

The term "C_2-C_n alkenyl" wherein n is an integer, as used herein, represents an olefinically unsaturated branched or linear group having from 2 to the specified number of carbon atoms and at least one double bond. Examples of such groups include, but are not limited to, 1-propenyl, 2-propenyl, 1,3-butadienyl, 1-butenyl, hexenyl, pentenyl, and the like.

The term "C_2-C_n alkynyl" wherein n is an integer refers to an unsaturated branched or linear group having from 2 to the specified number of carbon atoms and at least one triple bond. Examples of such groups include, but are not limited to, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl, 1-pentynyl, and the like.

The term "C_3-C_n cycloalkyl" wherein n = 8, represents cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl.

As used herein the term "aryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, benzyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl, and the like.

The term (C_5-C_8 alkyl)aryl refers to any aryl group which is attached to the parent moiety via the alkyl group.

The term "heterocyclic group" refers to a mono- or bicyclic carbocyclic ring system containing from one to three heteroatoms wherein the heteroatoms are selected from the group consisting of oxygen, sulfur, and nitrogen.

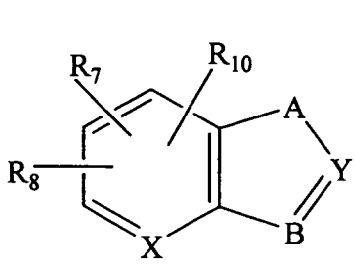
As used herein the term "heteroaryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings containing from one to three heteroatoms and includes, but is not limited to, furyl, thienyl, pyridyl and the like.

The term "bicyclic" represents either an unsaturated or saturated stable 7- to 12-membered bridged or fused bicyclic carbon ring. The bicyclic ring may be attached at any carbon atom which affords a stable structure. The term includes, but is not limited to, naphthyl, dicyclohexyl, dicyclohexenyl, and the like.

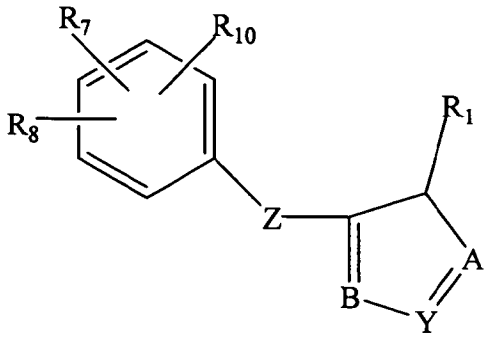
The term "pharmaceutically-acceptable salt" refers to salts which retain the biological effectiveness and properties of the S1P analogs of the present invention and which are not biologically or otherwise undesirable. In many cases, the S1P analogs of the present invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

Embodiments

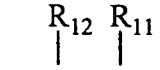
One aspect of the present invention is directed to novel compounds that inhibit the formation of HIV particles. In accordance with one embodiment an HIV inhibitor is provided wherein the compound has the general structure:

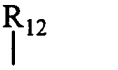
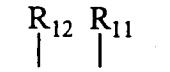


or



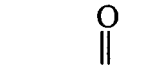
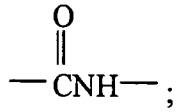
wherein

A is selected from the group consisting of N, CR_1, and  ;

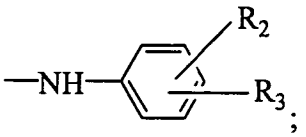
B is selected from the group consisting of N, S,  and  ;

Y is selected from the group consisting of Se, N, CH and CR_4;

X is selected from the group consisting of CH, CR_7 and N;

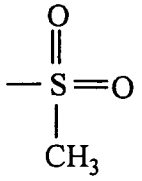
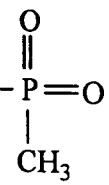
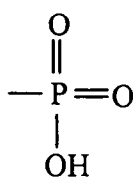
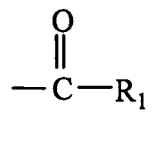
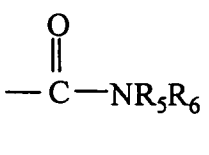
Z is selected from the group consisting of C_0-C_4 alkyl,  and  ;

R_1 and R_{12} are independently selected from the group consisting of H, halo, CO, C_1-C_4 alkyl, C_1-C_4 haloalkyl, NR_5R_6, optionally substituted C_5-C_6 aryl and

 ;

R_2 and R_3 are independently selected from the group consisting of H, halo, hydroxy and C_1-C_4 alkyl;

R_4 is selected from the group consisting of H, halo, hydroxy, C_1-C_4 alkyl and C_1-C_4 haloalkyl;

 ,  ,  ,  and 

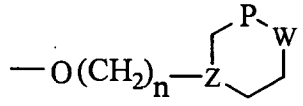
R_5 and R_6 are independently selected from the group consisting of H and C_1-C_4 alkyl;

R_7 and R_8 are independently selected from the group consisting of H, halo, hydroxy, C_1-C_4 alkyl, C_1-C_4 alkoxy, -C(O)CH_3, -NHC(O)CH_3 and -O(C_1-C_4 alkyl)(C_5-C_6 heterocyclic) or R_7 and R_8 together with the atoms to which they are attached form an optionally substituted C_5-C_6 aryl, wherein the aryl ring is optionally substituted with halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl(C_5-C_6 aryl) and -O(C_1-C_4 alkyl)(C_5-C_6 heterocyclic);

R_{10} is selected from the group consisting of H, halo, hydroxy, C_1-C_4 alkyl, C_1-C_4 haloalkyl, acyl, -CN, C_1-C_4 alkoxy, -NHC(O)CH_3, optionally substituted C_5-C_6 aryl, optionally substituted C_5-C_6 heteroaryl and -O(C_1-C_4 alkyl)(C_5-C_6 heterocyclic); and

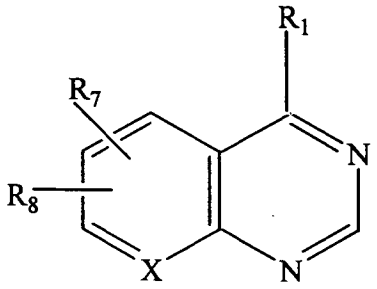
R_{11} is selected from the group consisting of H, halo, hydroxy, C_1-C_4 alkyl, C_1-C_4 alkoxy and -CN.

In one embodiment Y is CR_4, R_7 is H or C_1-C_4 alkoxy, R_8 is halo or

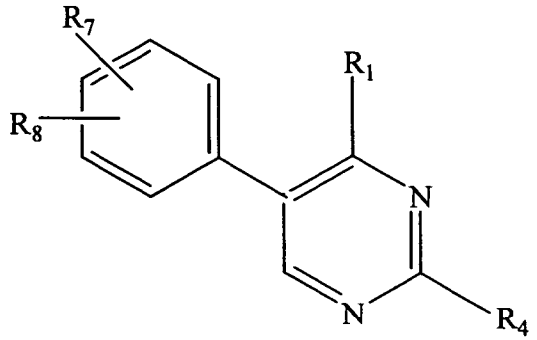


, wherein n is an integer ranging from 1-5, and P, W and Z are independently selected from the group consisting of O, S, CH_2 and NH.

In another embodiment a compound is provided wherein the compound has the general structure:



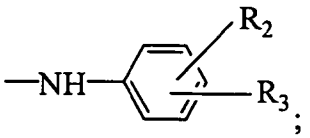
or



wherein

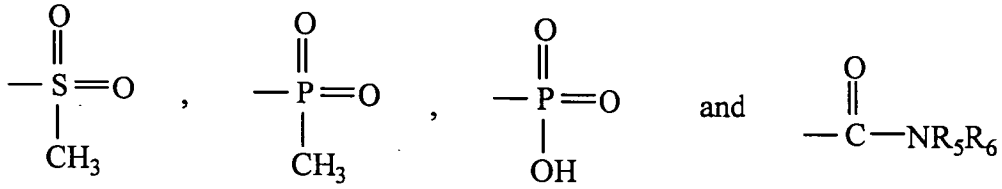
X is selected from the group consisting of CH and N;

R_1 is selected from the group consisting of H, NR_5R_6 and



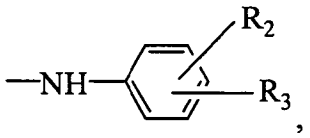
R_2 and R_3 are independently selected from the group consisting of H, halo, hydroxy and C_1-C_4 alkyl;

R_4 is selected from the group consisting of H, halo, hydroxy and C_1-C_4 alkyl,



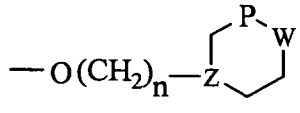
R_5 and R_6 are independently selected from the group consisting of H and C_1-C_4 alkyl;

R_7 and R_8 are independently selected from the group consisting of H, halo, hydroxy, C_1-C_4 alkyl, C_1-C_4 alkoxy, -$NHC(O)CH_3$ and -$O(C_1$-C_4 alkyl)(C_5-C_6 heterocyclic) or R_7 and R_8 together with the atoms to which they are attached form an optionally substituted C_5-C_6 aryl, wherein the aryl ring is optionally substituted with halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl(C_5-C_6 aryl) and -$O(C_1$-C_4 alkyl)(C_5-C_6 heterocyclic). In one embodiment R_1 is NR_5R_6 or



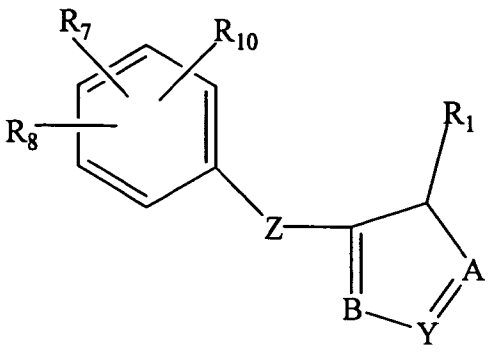
R_7 is H or C_1-C_4 alkoxy, and

R_8 is halo or

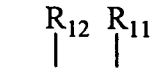


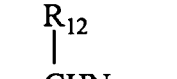
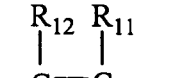
, wherein n is an integer ranging from 1-5, and P, W and Z are independently selected from the group consisting of O, S, CH_2 and NH. Another embodiment of the invention is directed to the compounds of Fig. 2.

In accordance with one embodiment a compound is provided having the general formula

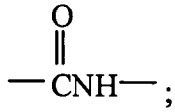


wherein

A is selected from the group consisting of N, and  ;

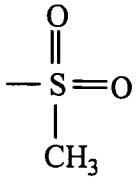
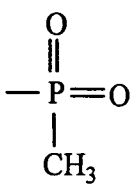
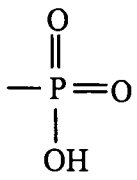
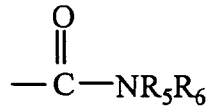
B is selected from the group consisting of N, S,  and  ;

Y is selected from the group consisting of N, CH and CR_4;

Z is selected from the group consisting of C_0-C_4 alkyl, and  ;

R_1 and R_{12} are independently selected from the group consisting of H, halo, C_1-C_4 alkyl, C_1-C_4 haloalkyl and NR_5R_6;

R_4 is selected from the group consisting of H, halo, hydroxy and C_1-C_4 alkyl, C_1-C_4 haloalkyl;

 ,  ,  and 

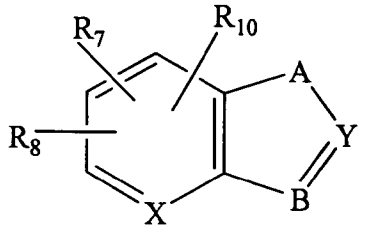
R_5 and R_6 are independently selected from the group consisting of H and C_1-C_4 alkyl;

R_7 and R_8 are independently selected from the group consisting of H, halo, hydroxy, C_1-C_4 alkyl and C_1-C_4 alkoxy;

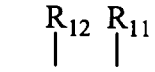
R_{10} is H; and

R_{11} is selected from the group consisting of H, halo, hydroxy, C_1-C_4 alkyl, C_1-C_4 alkoxy and -CN.

In accordance with one embodiment a compound is provided having the general formula



wherein

A is selected from the group consisting of CR_1, and  ;

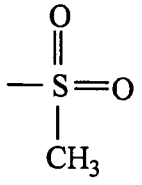
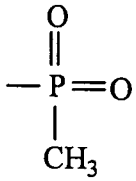
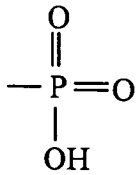
B is selected from the group consisting of N and S;

Y is selected from the group consisting of N, CH and CR_4;

X is selected from the group consisting of CH and N;

R_1 and R_{12} are independently selected from the group consisting of H, halo, C_1-C_4 alkyl, C_1-C_4 haloalkyl and NR_5R_6;

R_4 is selected from the group consisting of H, halo, hydroxy and C_1-C_4 alkyl, C_1-C_4 haloalkyl;

 ,  ,  and 

R_5 and R_6 are independently selected from the group consisting of H and C_1-C_4 alkyl;

R_7 and R_8 are independently selected from the group consisting of H, halo, hydroxy, C_1-C_4 alkyl, C_1-C_4 alkoxy, -$NHC(O)CH_3$ and -O(C_1-C_4 alkyl)(C_5-C_6 heterocyclic) or R_7 and R_8 together with the atoms to which they are attached form an optionally substituted C_5-C_6 aryl, wherein the aryl ring is optionally substituted with halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl(C_5-C_6 aryl) and -O(C_1-C_4 alkyl)(C_5-C_6 heterocyclic);

R_{10} is selected from the group consisting of H, halo, hydroxy, C_1-C_4 alkyl, C_1-C_4 alkoxy, and C_1-C_4 haloalkyl; and

R_{11} is selected from the group consisting of H, halo, hydroxy, C_1-C_4 alkyl, C_1-C_4 alkoxy and -CN.

The present invention is also directed to pharmaceutical compositions comprising the HIV inhibitory compounds of the present invention. More particularly, such compounds can be formulated as pharmaceutical compositions using standard pharmaceutically acceptable carriers, fillers, solublizing agents and stabilizers known to those skilled in the art. Pharmaceutical compositions comprising the present compounds are administered to an individual in need thereof by any number of routes including, but not limited to, topical, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In accordance with one embodiment, a method of treating HIV is provided. The method comprises the steps of administering a composition comprising an HIV inhibitory compound of the present invention to a patient in need thereof.

Example 1

Compounds were identified using a primary screening assay that involved the use of a cell line (5BD.1) that was continuously expressing HIV virus-like particles. To measure inhibition, supernatants containing HIV virus-like particles were obtained from the tissue culture assay described herein. The amount of HIV particles in the assay was then measured using the ELISA Assay that is also described below. The final ELISA data from the screening of approximately 40,000 compounds (commercially available from SPECS and BioSPECS, Rijswijk, The Netherlands) is shown in Figs 1A-I.

Compounds that reduced HIV particle formation by at least 50% as measured by these assays were screened further in the 3 and 6 point dose response assays and toxicity assays.

Primary Screening Assays

Tissue Culture Assay:

1. 5BD.1 cells were passaged in 2 T225 flasks in medium (IMDM/10% FCS/0.2 mg/ml HygromycinB/1.5 mg/ml G418/0.05 mg/ml gentamycin). Cells were trypsinized and harvested from 2 90% confluent flasks with 28.4x106 cells recovered.
2. 4500 cells per well were plated into columns of tissue culture treated clear 384 well plates in 40 ul per well of medium.
3. The plate was placed into the incubator for one hour.
4. Compounds were diluted from 2 ul of 1 mM DMSO stocks in 384 well polypropylene plates by adding 38 ul per well of medium.
5. 10 ul of each diluted compound was transferred to the cell plates.
6. The plates were then incubated overnight for 16 hours.
7. In the morning of the next day the plates were aspirated on a plate washer.
8. 40 ul of fresh medium was added to each well followed by 10 ul of diluted as in step 5.
9. The plates were then incubated for 8 hours.
10. 25 ul of supernatant per well from all wells was added to the plates coated and blocked below as described in step 14 below.

p24 ELISA Assay

11. Dilute primary antibody to 4 ug/ml in DPBS without calcium and magnesium, add 25 ul per well of a 384 well Maxisorp plate, incubate overnight at 4oC.
12. Aspirate coating solution, block for 30-60 minutes with 100 ul ELISA buffer (4 mg/ml BSA, 0.01% Tween20 in DPBS without calcium and magnesium).
13. Wash plates 2X.
14. Add 25ul of supernatant from step 10 above.
15. Add 10 ul of a 1:250 dilution of biotinylated secondary antibody in 25% lysis buffer/ELISA buffer.
16. Incubate overnight in the refrigerator.
17. Wash plates 3X.

18. Add 25 uL/well of a 1:10,000 dilution of detection SA-HRP. Incubate at room temp for 30 minutes.
19. Wash plates 3X. Add 25 uL/well of TMB substrate solution to all wells and develop for approximately 5 minutes until blue.
20. Stop the reaction with 25 uL/well 0.18M sulfuric acid.
21. Read plate at 450nm.

Compounds that gave 50% reduction in optical density as measured by this assay were identified and selected in this manner. The structure of each of twelve of these compounds being claimed in this disclosure is shown in Fig 2. (192 were selected originally).

Dose Response Assays

Each of the 192 compounds were then subjected to a 3 point dose response assay using the cell line. 33 compounds scored positive in the three dose response and were then subjected to a 6 point dose response. The data from these assays for the 12 compounds being disclosed here are given in figures 3 and 4. The two assays were performed as follows:

3 and 6 point Dose Response Assays

Tissue Culture

1. 5BD.1 cells were carried in 2 T175 flasks in medium (IMDM/10% FCS/0.2 mg/ml HygromycinB/1.5 mg/ml G418/0.05 mg/ml gentamycin). Cells were trypsinized and harvested from a 90% confluent flask.
2. 20,000 cells per well were plated into rows A-G of 8 tissue culture treated clear 96-well plates in 135 ul per well of medium without G418 (assay medium). 135 ul of medium only was added to row H.
3. The plates were placed into the incubator for one hour.
4. Compounds were serially diluted 1:3 two times from 1 mM DMSO stocks in DMSO in 96 well polypropylene plates. 8 ul of the DMSO solutions were transferred to another plate and 72 ul of assay medium was added.
5. 15 ul of each diluted compound was transferred to the cell plates in duplicate with the high (10 uM final concentration in rows A-B, 3 uM concentration in rows C-D, 1 uM concentration in rows E-F and DMSO only in rows G-H).
6. The plates were then incubated overnight for 16 hours.
7. In the morning of the next day the medium was removed by hand from all wells.
8. 135 ul of fresh assay medium was added to each well followed by 15 ul of diluted compounds as described in step 5.
9. The plates were then incubated for 24 hours.
10. 50 ul of supernatant per well from all wells was harvested and added to the ELISA plates in step 15 below.

p24 ELISA Assay

11. Dilute primary antibody to 4 ug/ml in DPBS without calcium and magnesium, add 50 ul per well of a 96 well Maxisorp plate, incubate overnight at 4oC.
12. Aspirate coating solution, block for 30-60 minutes with 200 ul ELISA buffer (4 mg/ml BSA, 0.01% Tween20 in DPBS without calcium and magnesium).
13. Wash plates 2X.
14. Add 50 ul of the supernatants from step 10 above.

15. Add 15 ul of a 1:750 dilution of biotinylated secondary antibody in 40% lysis buffer/ELISA buffer.
16. Incubate 2 hours room temp with shaking.
17. Wash plates 3X.
18. Add 50 uL/well of a 1:10,000 dilution of detection SA-HRP. Incubate at room temperature with shaking for 30 minutes.
19. Wash plates 3X. Add 50 uL/well of TMB substrate solution to all wells and develop for approximately 15 minutes until blue.
20. Stop the reaction with 50 uL/well 0.18M sulfuric acid.
21. Read plate at 450nm.

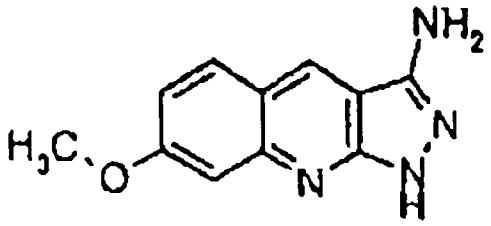
Toxicity Assays:

MTS-Assay

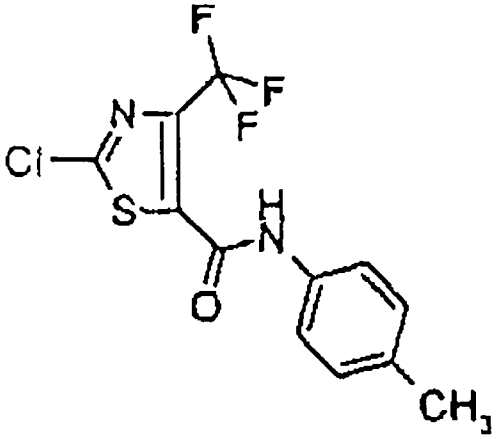
MTS-based toxicity assays were performed in parallel to the 3 and 6 point dose response assays. The assay uses MTS a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS(b)] and an electron coupling reagent (phenazine ethosulfate; PES) and was performed according to the directions of its manufacturer Promega, Madison Wisconsin. (see attached protocol Technical Bulletin #245 from Promega). The MTS assay data for the 12 compounds disclosed here after two days of incubation with 5BD.1 cells are shown in figures 5 and 6 and after incubation for 5 days with MT-4 T cells are shown in Figure 7.

Example 2

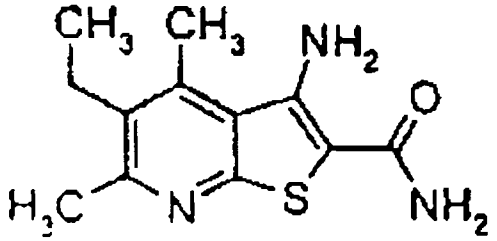
Additional compounds suitable for use in accordance with the present invention are included on the following pages:



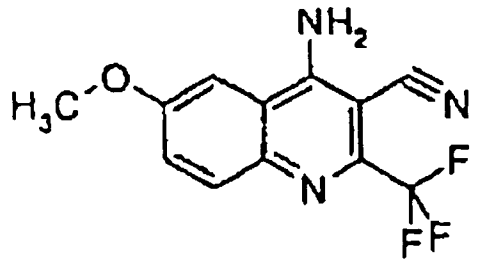
Message Name: 89246
Speks Name: AG-690/40701421
Chemical Name: 7-methoxy-1H-pyrazolo[3,4-b]quinolin-3-ylamine



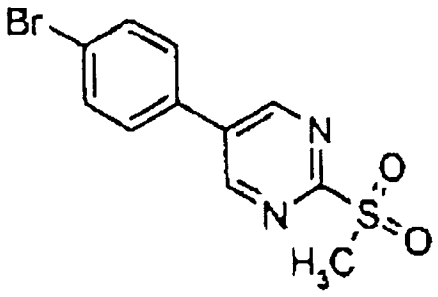
Message Name: 91161
Speks Name: AP-501/40888738
Chemical Name: 2-chloro-N-(4-methylphenyl)-4-(trifluoromethyl)-1,3-thazole-5-carboxamide



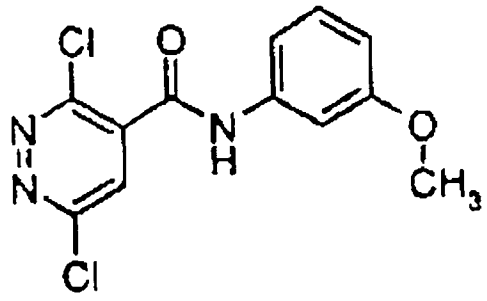
Message Name: 103833
Speks Name: AE-848/34435011
Chemical Name: 3-amino-5-ethyl-4,6-dimethylthieno[2,3-b]pyridine-2-carboxamide



Message Name: 104366
Speks Name: AG-687/25019010
Chemical Name: 4-amino-6-methoxy-2-(trifluoromethyl)-3-quinolinecarbonitrile

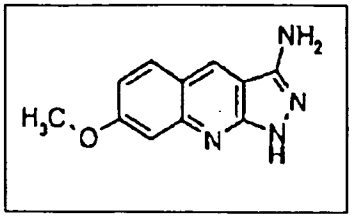


Message Name: 107129
Speks Name: AC-907/25005415
Chemical Name:

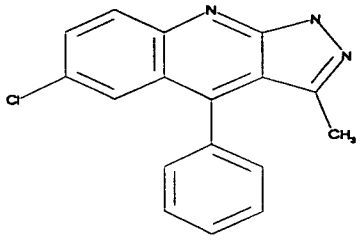


Message Name: 107740
Speks Name: AF-399/40653810
Chemical Name: 3,6-dichloro-N-(3-methoxyphenyl)-4-pyridazinecarboxamide

89246 AG-690/40701421



AH-034/34961017



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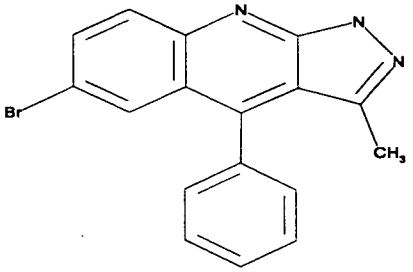

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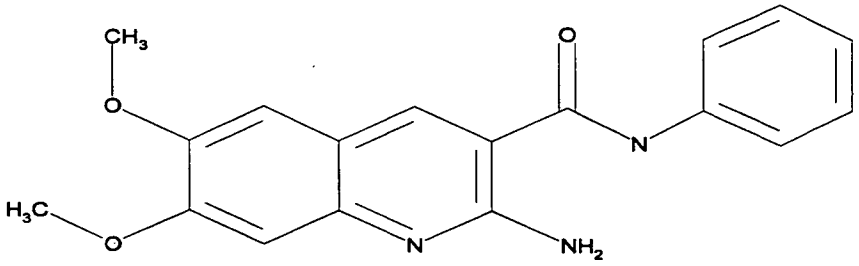

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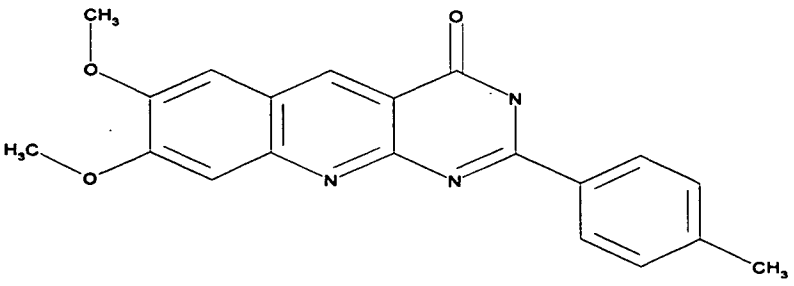
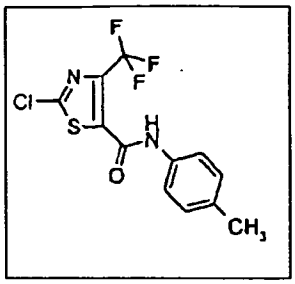
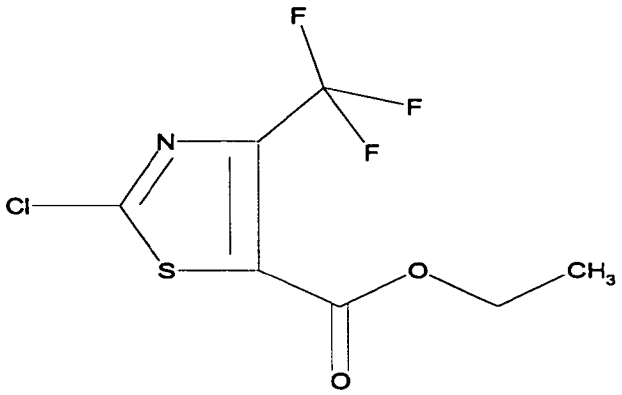


91161 AP-501/40888738



AP-501/40804729



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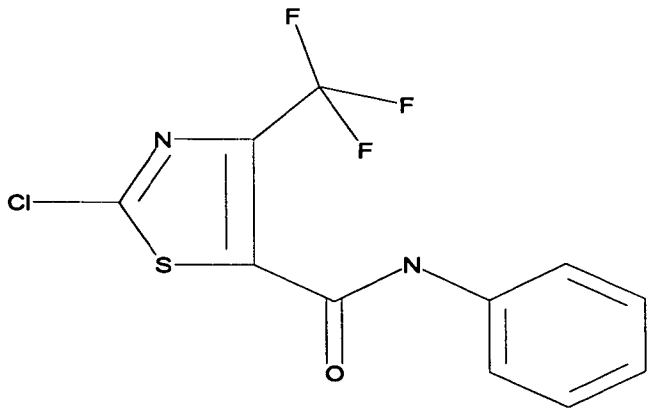
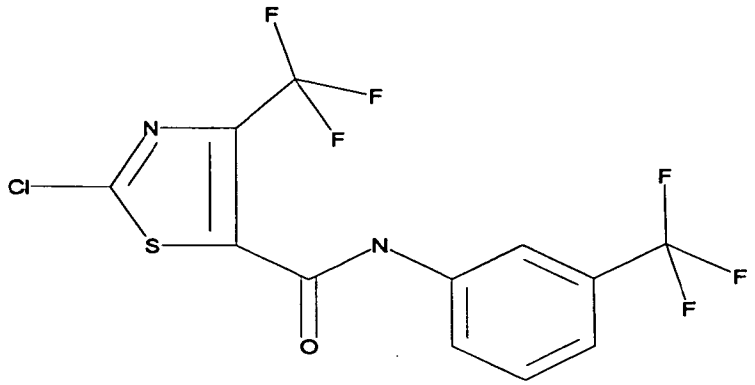
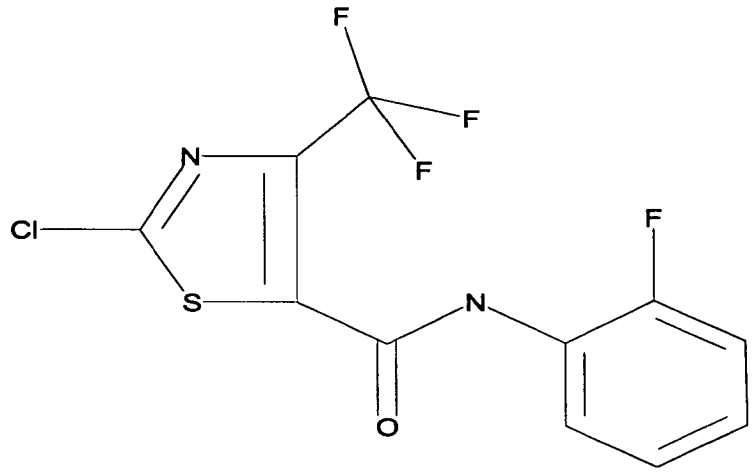


AP-501/40804756



AP-501/40804757



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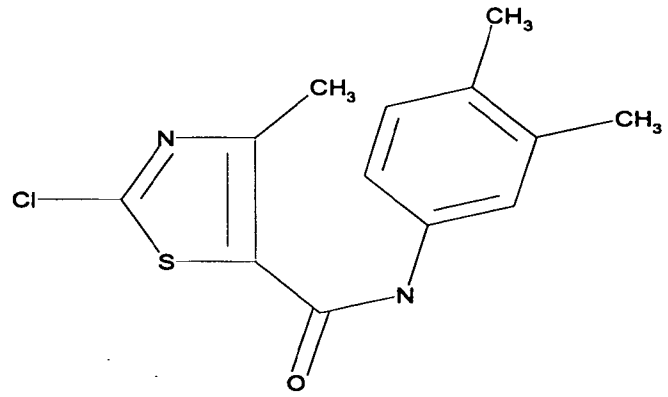
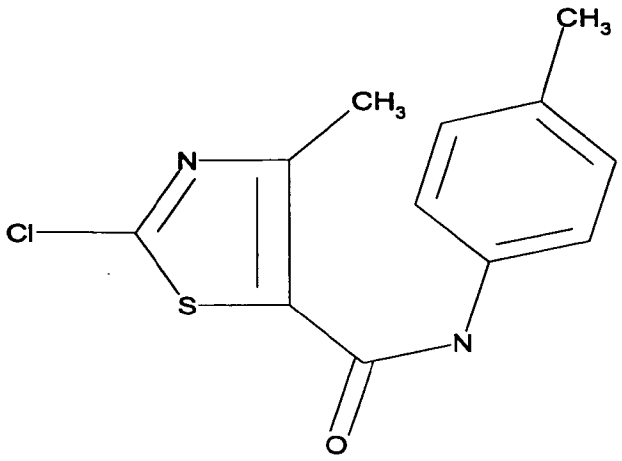


AP-501/40888736



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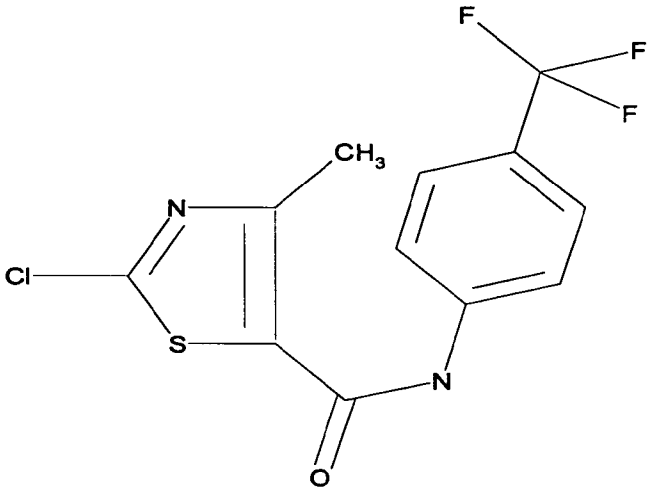

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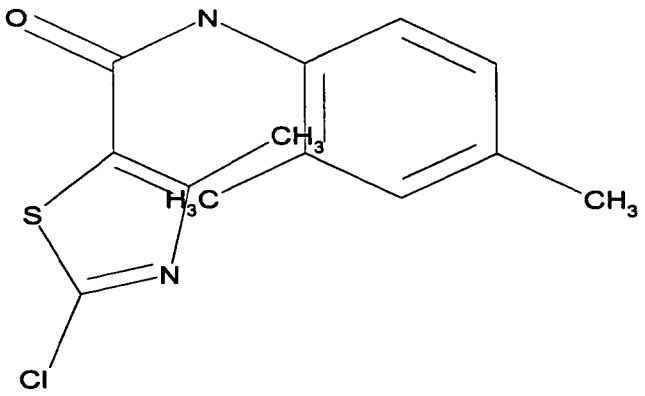

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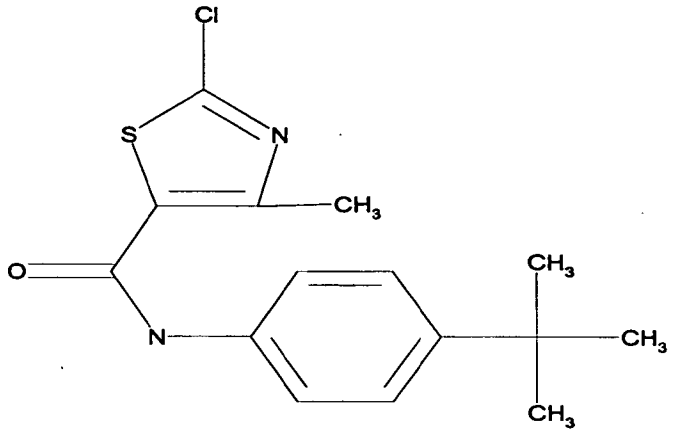

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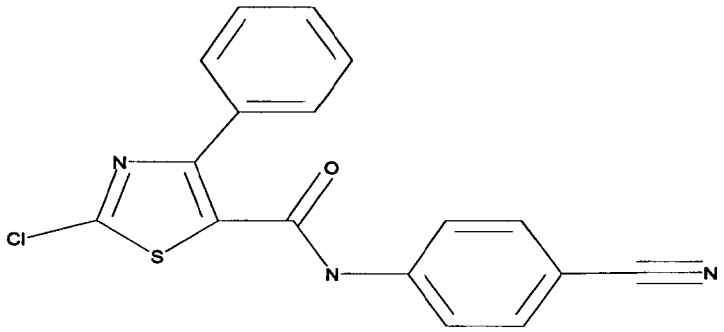

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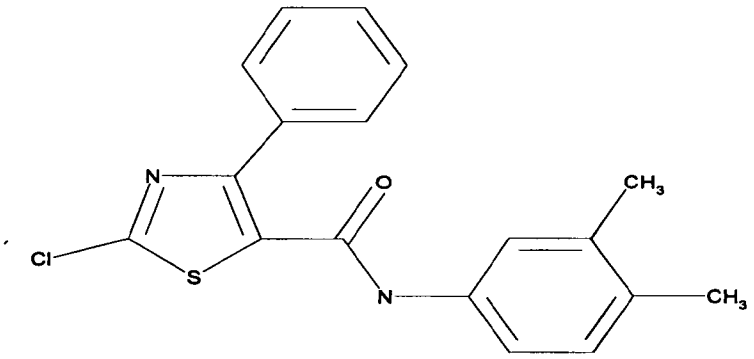

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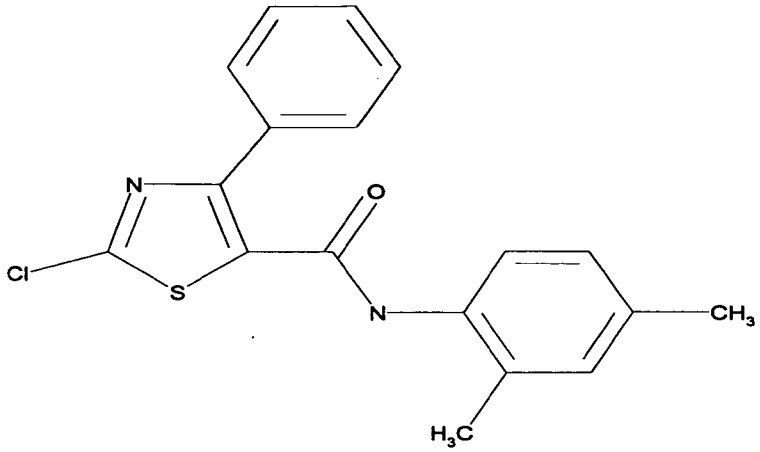

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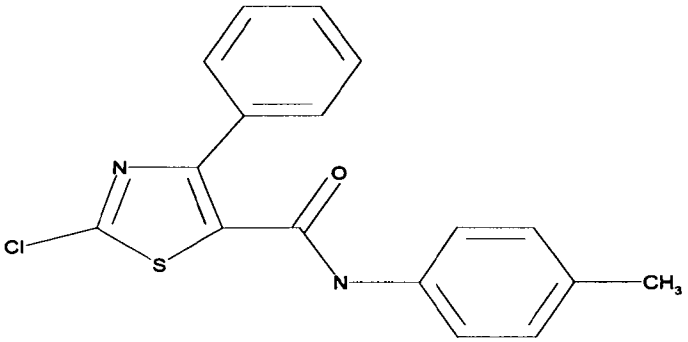

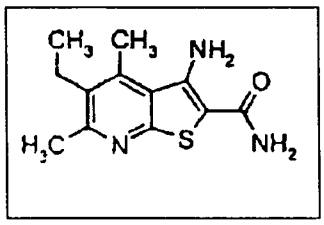

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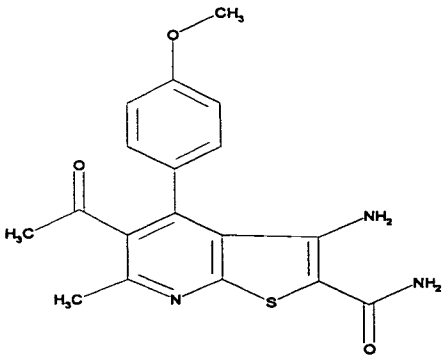

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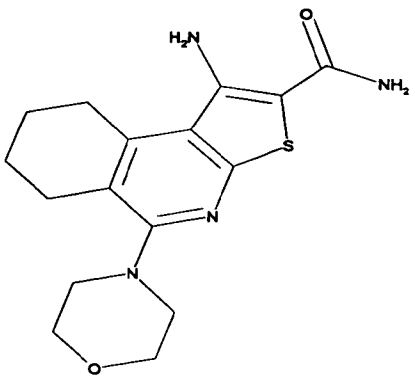

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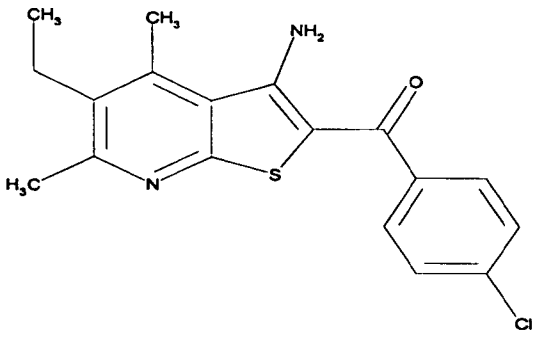

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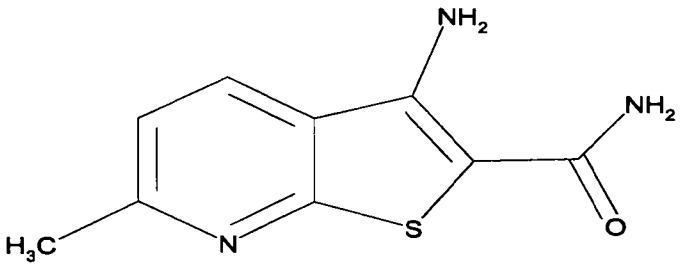

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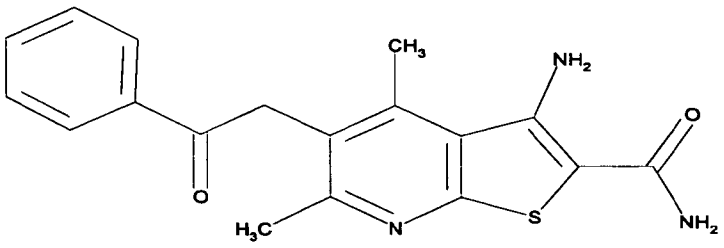

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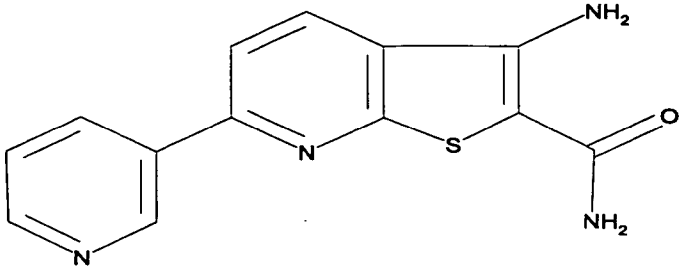

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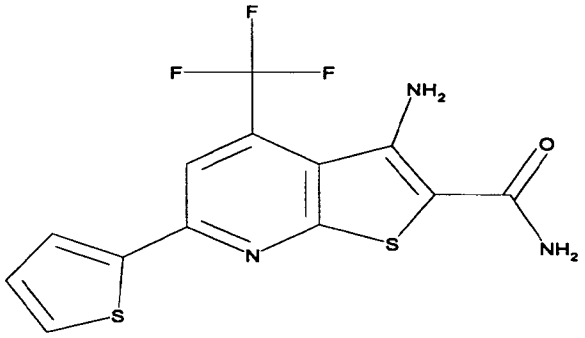

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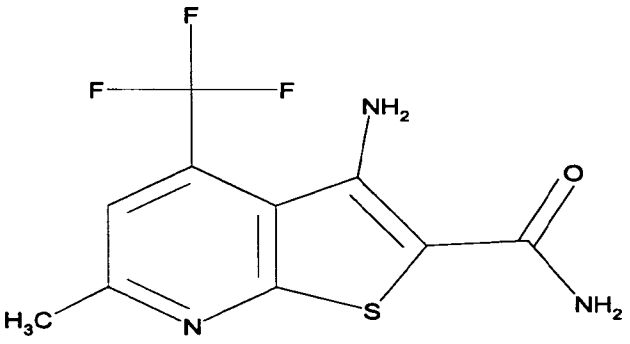

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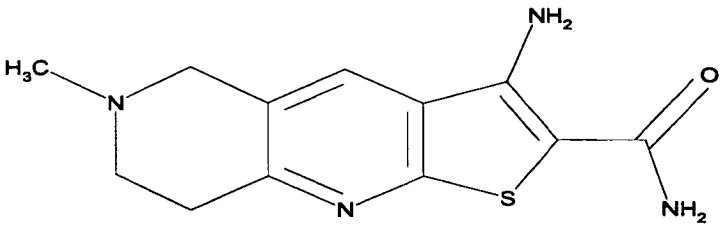

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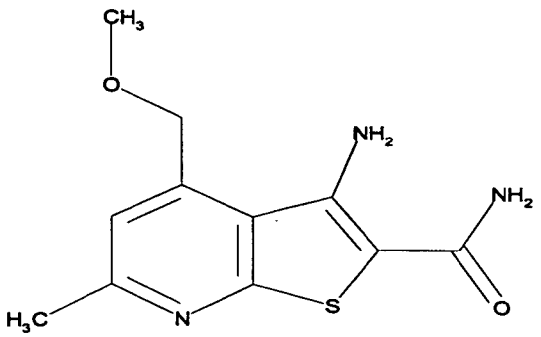

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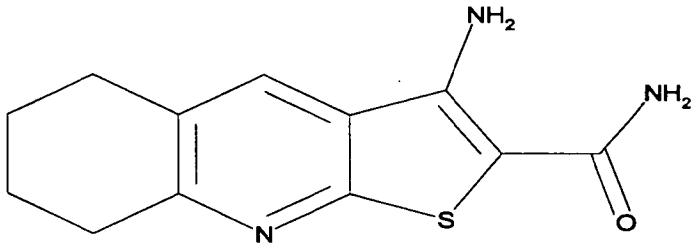

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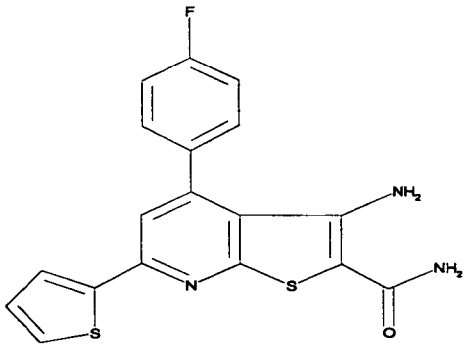

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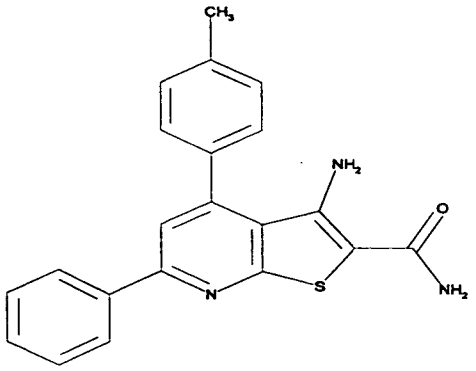

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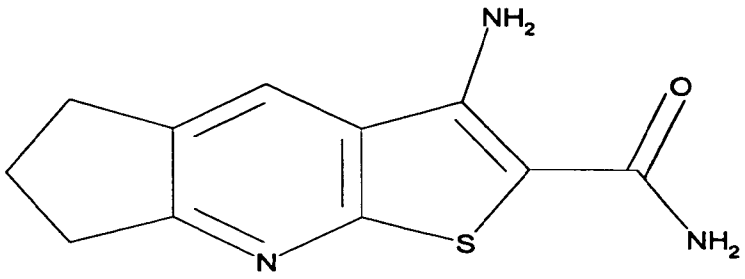

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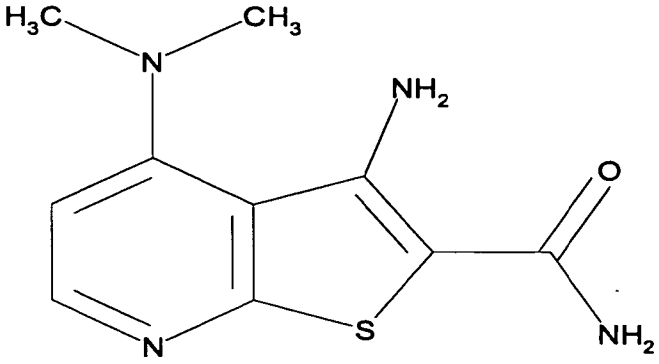

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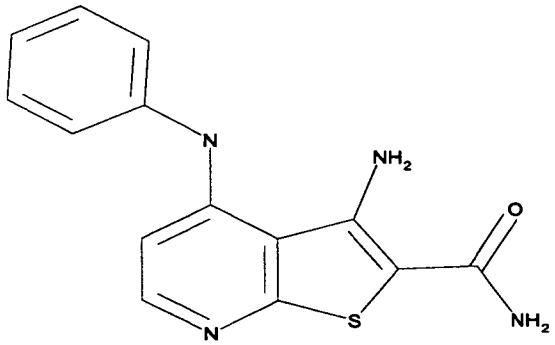

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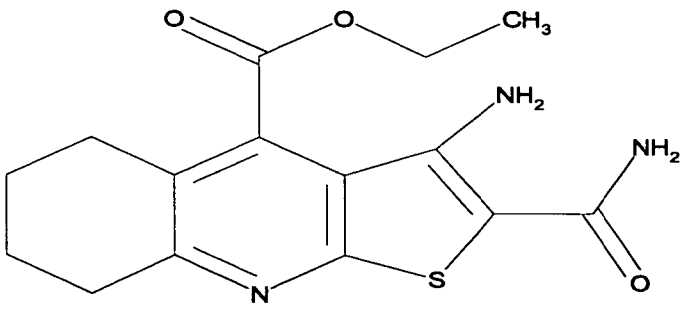

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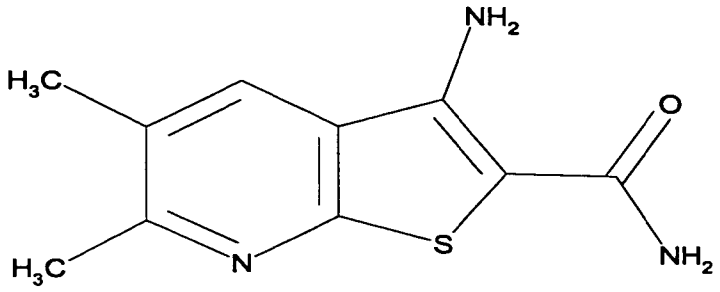

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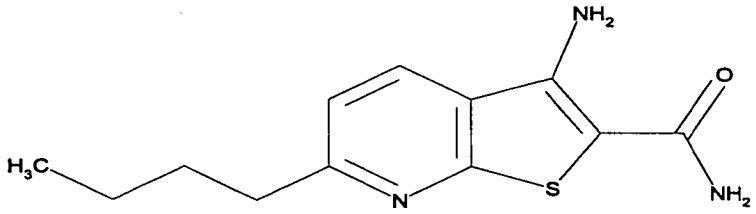

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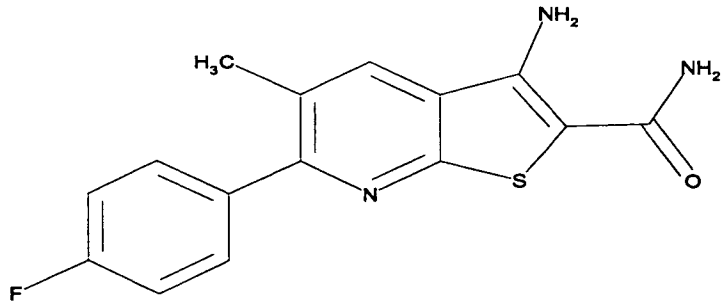

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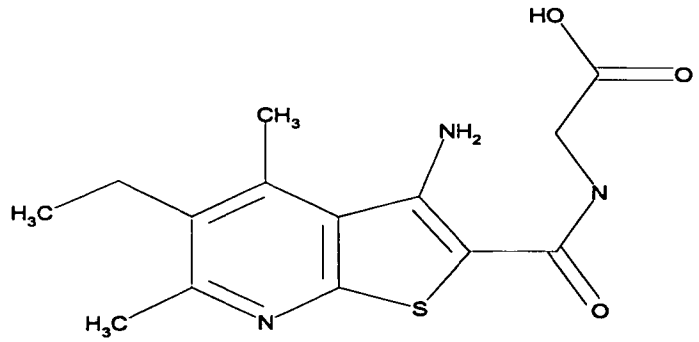

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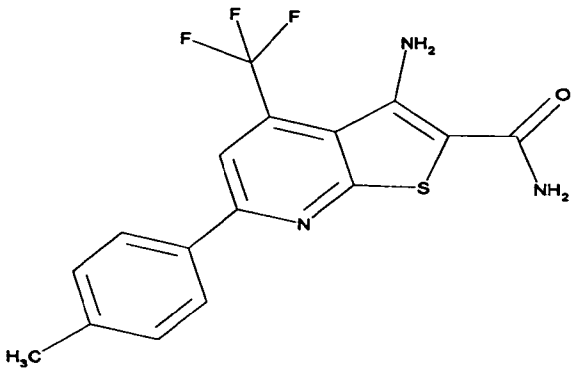

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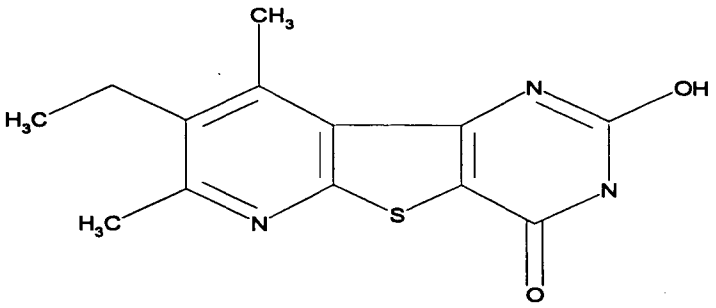

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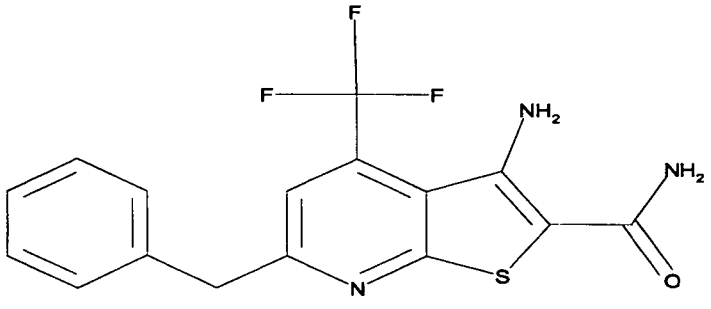

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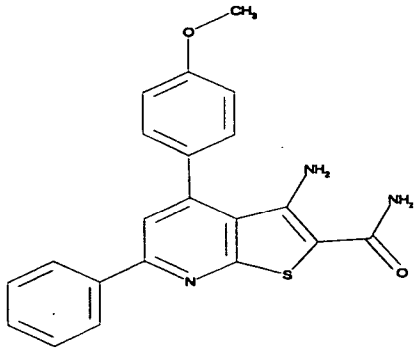

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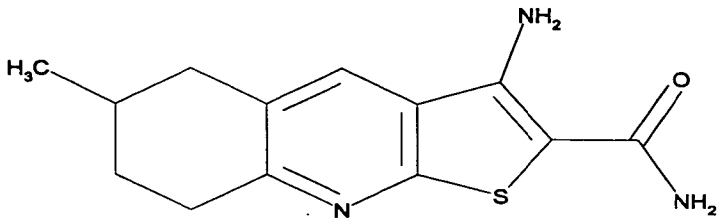

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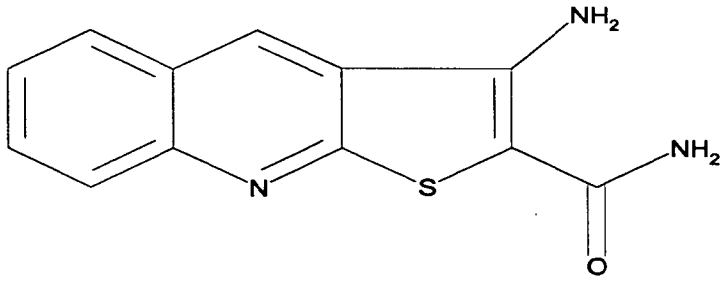

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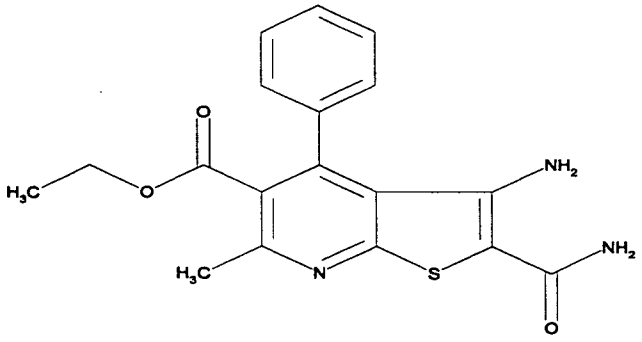

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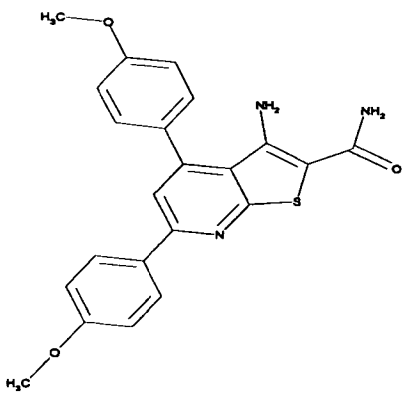

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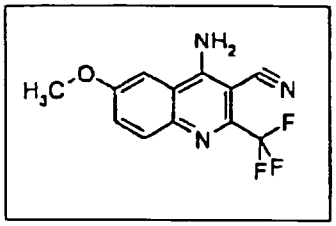

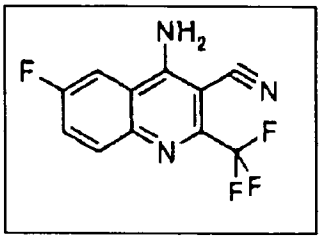

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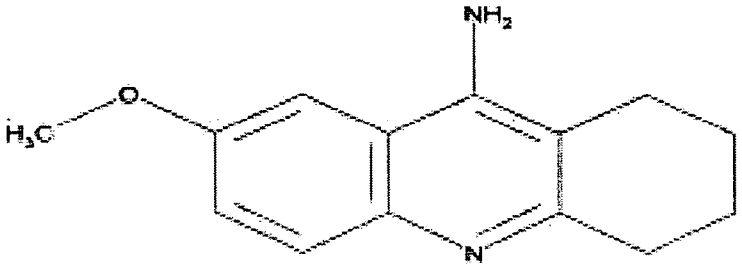

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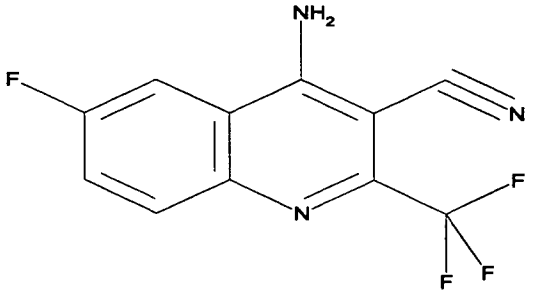

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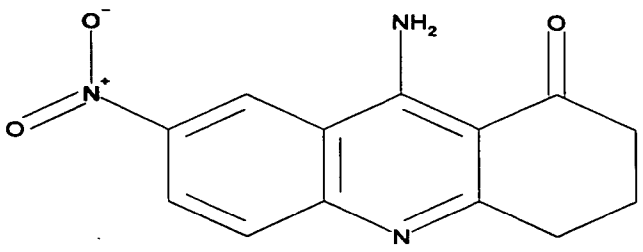

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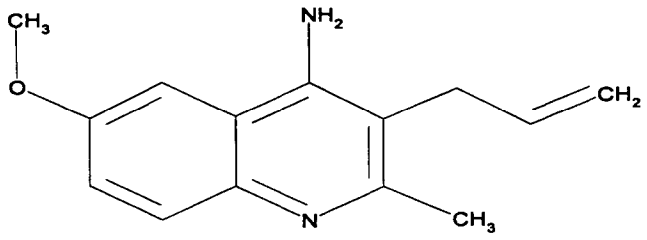

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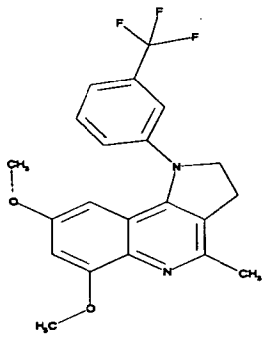

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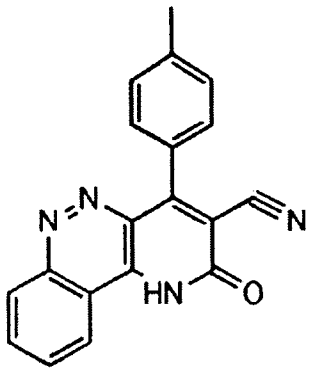

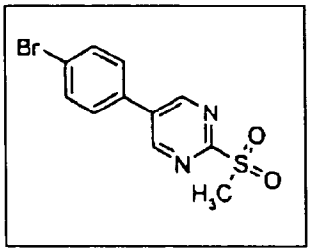

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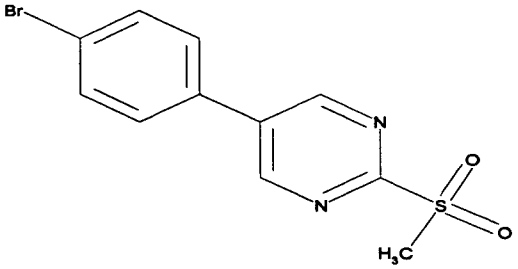

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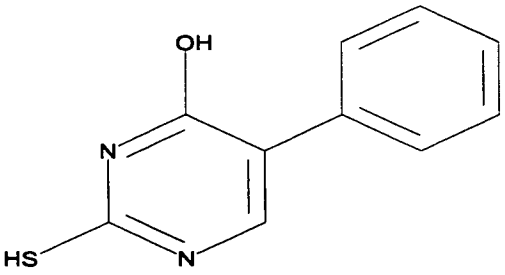

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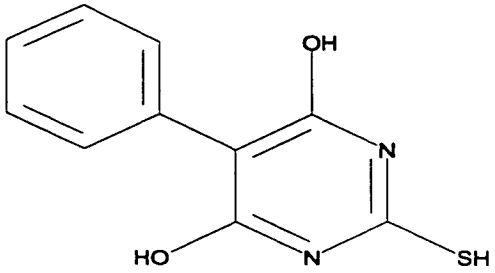

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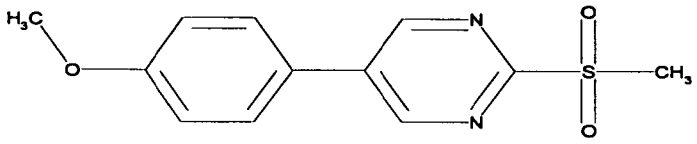

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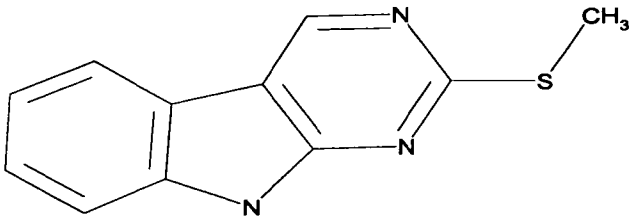

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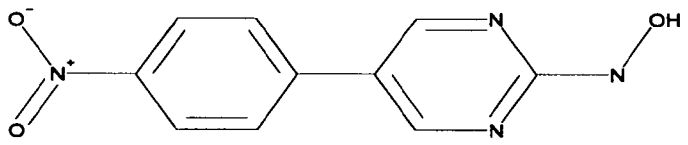

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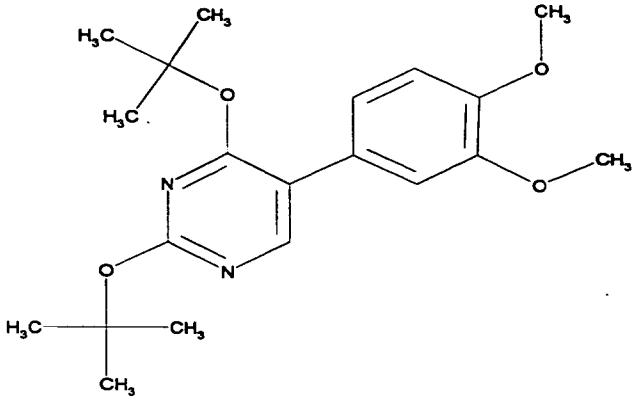

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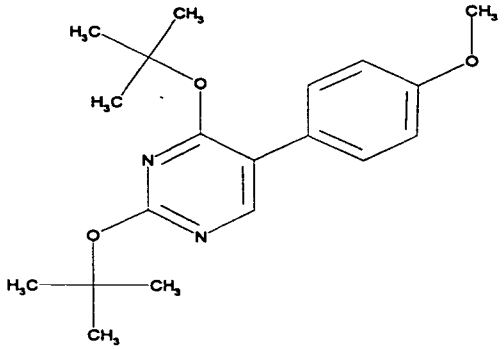

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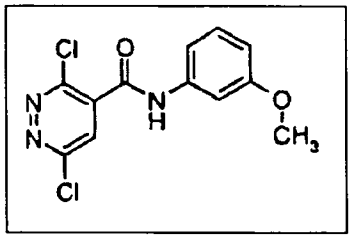

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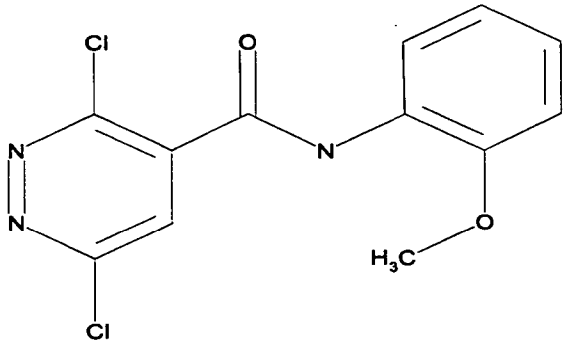

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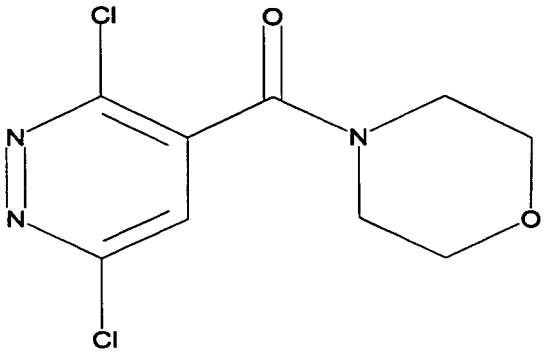

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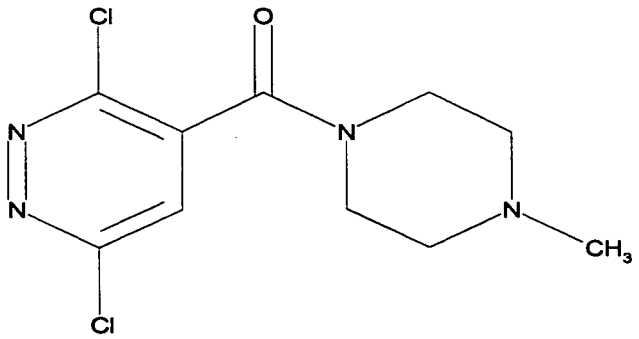

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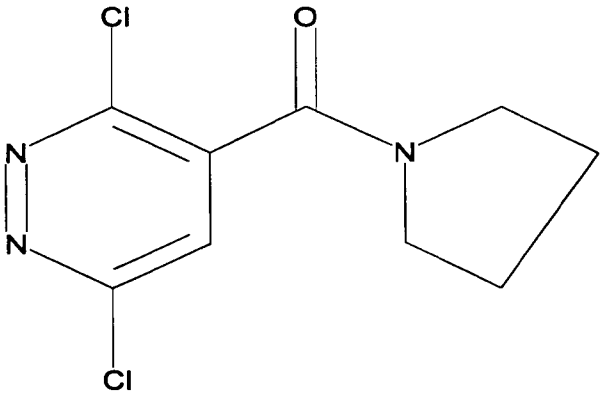

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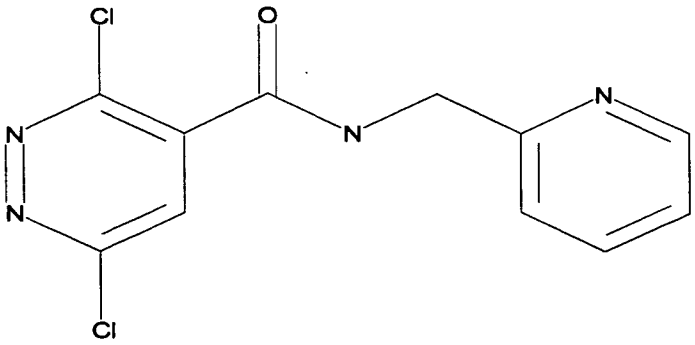

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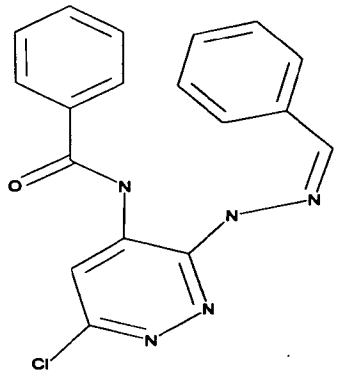


Rev Assay Description

The HIV-1 Rev assay is based upon the use of a reporter for detecting whether drug candidates are capable of inhibiting the function of the viral Rev protein. The reporter used for these assays is the Renilla Luciferase protein, which is an enzyme that produces detectable light when mixed with certain chemicals. For this assay, a cell line has been developed in which the production of the reporter by the cells requires the function of HIV-1 Rev. Therefore, if a drug candidate inhibits the function of Rev, it decreases the amount of the reporter produced by the cells. By using Renilla Luciferase as the reporter, the inhibition of Rev is easily detected as a decrease in the amount of light produced by the cells when mixed with the appropriate chemicals. Furthermore, the cell line for this assay has been engineered to use a second similar reporter (Firefly Luciferase) that detects whether drug candidates are toxic. By using this two reporter, or Dual-Luciferase, approach, compounds that specifically inhibit HIV-1 Rev can be identified. More detailed information about the assay is provided in the table below.

Parameter	HIV-1 Rev Assay
Assay Principal	HIV-1 Rev-dependent Luciferase reporter expression construct engineered into a stable cell line.
Reference for Assay Principal	Hope et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:7787-7791.
Cell Line	HeLa (cervix; epithelial; adenocarcinoma)
Genetic Modifications	Stably integrated bicistronic expression construct for both the HIV-1_{MB} Rev gene and Firefly Luciferase gene under the control of a single Tet-Off promoter; Stably integrated HIV-1_{SF2} Rev-dependent Renilla Luciferase reporter expression construct for monitoring Rev function.
Cell Line Maintenance Media	DMEM supplemented with 10% Tet-Free FBS, L-Glutamine, Pen/Strep, Geneticin (G418), Hygromycin B and Puromycin.
Passage	Trypsonized and split 1:5 twice weekly; Fresh cells thawed from LN_2 storage routinely to minimize loss of reporter gene expression upon serial passage.
Assay Media	DMEM supplemented with 10% Tet-Free FBS, L-Glutamine, Pen/Strep.
Standard Assay Conditions	• $2x10^4$ cells/well; 96 well format. • Drugs tested at 6 concentrations in triplicate assay wells. • Cells and drug added to wells in 200 µL total volume. • Plates incubated for **24 hours** at 37°C in humidified 5% CO_2 atmosphere. • Drugs/media removed, cells lysed and assayed for Dual-Luciferase® reporter expression according to manufacturers kit instructions (Promega, Madison, WI).
Endpoint Detection	• **Firefly Luciferase:** luminescence (relative light units) for detection of Tat expression and compound cytotoxicity/non-specificity • **Renilla Luciferase:** luminescence (relative light units) for detection of compound inhibition of Rev function
Assay Controls	• **Doxycycline:** turns off Tet-Off promoter to shut down both Firefly and Renilla Luciferase Expression and verify assay system functioning properly • **Leptomycin B:** Positive control; inhibitor of hCRM1 mediated Rev nuclear export. • **Other positive controls:** Currently being identified and tested.
Data Analysis	Calculations of compound efficacy for inhibiting Rev function as 50% inhibition of Renilla Luciferase (IC_{50}), compound toxicity/non-specificity as 50% inhibition of Firefly Luciferase (TC_{50}) and Therapeutic Index ($TI = TC_{50}/IC_{50}$).

Compound Information

Compound	Stock Concentration Provided (mM)	High-Test Concentration Used (μM)
49611	25.4	63.5
73497	36.25	90.625
74377	16.7	41.75
74378	17.3	43.25
75168	10.7	26.75
89246	21.8	54.5
91161	15.8	39.5
103833	15.04	37.6
104366	31.8	79.5
107129	21.2	53.0
107740	11.7	29.25
109020	16.3	40.75

Compounds were tested using the maximum high-test concentration possible based on the supplied stocks. Compounds were prepared at a 2X high-test concentration by combining drug stock with tissue culture media at a ratio of 5 μL of drug to 995 μL of media. This 2X high-test sample was subsequently serially diluted in tissue culture media using ½-log dilutions. This series of 2X concentrated drug was subsequently diluted 1:1 by combining with an equal volume of cells in media in the 96-well plates used for the assay.

Results for Rekosh/Hammarskjöld Candidate Rev Inhibitors

Compound	Setup Date	IC_{90} (μM)	IC_{50} (μM)	TC_{50} (μM)	TI (TC_{50}/IC_{50})	Comments
49611	3/17/04	16.1	1.43	3.61	2.52	Activity parallels toxicity
73497	3/17/04	61.4	8.99	51.6	5.74	Activity parallels toxicity
74377	3/17/04	>41.8	11.2	>41.8	>3.73	Activity parallels toxicity
74378	3/17/04	>43.2	11.0	33.0	3.00	Activity parallels toxicity
75168	3/17/04	13.0	2.42	7.91	3.27	Activity parallels toxicity
89246	3/17/04	2.88	0.48	8.41	17.52	**Specific anti-Rev activity**
91161	3/17/04	27.0	9.73	>39.5	>4.06	**Specific anti-Rev activity**
103833	3/17/04	6.75	1.07	>37.6	>35.14	**Specific anti-Rev activity**
104366	3/17/04	7.67	2.24	17.2	7.68	**Specific anti-Rev activity**
107129	3/17/04	28.4	7.48	30.9	4.13	**Specific anti-Rev activity**
107740	3/17/04	22.2	8.00	18.5	2.31	Activity parallels toxicity
109020	3/17/04	32.5	8.98	>40.8	>4.54	**Specific anti-Rev activity**
Leptomycin B	3/17/04	0.0227	0.00822	0.0200	2.43	Control 1
Leptomycin B	3/17/04	0.0230	0.00817	0.0192	2.35	Control 2
Leptomycin B	3/17/04	0.0206	0.00776	0.0214	2.76	Control 3
Leptomycin B	3/17/04	0.0210	0.00752	0.0209	2.78	Control 4

NOTE: "Toxicity" in this assay reflects non-specific compound activity in the assay as demonstrated by a reduction in the Firefly Luciferase signal, which is Rev-independent. This does not necessarily correlate with cell killing.

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Compound 49611

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (μM)	0.00	0.20	0.6	2.0	6.4	20.1	63.5
SAMPLE 1	0.1270	0.1340	0.1411	0.0662	0.0350	0.0075	0.0000
SAMPLE 2	0.1278	0.1127	0.0774	0.0440	0.0270	0.0085	0.0000
SAMPLE 3	0.1464	0.1146	0.0830	0.0485	0.0432	0.0087	0.0000
MEAN	0.1337	0.1205	0.1005	0.0529	0.0351	0.0083	0.0000
% CONTROL	100.0	90.1	75.2	39.6	26.2	6.2	0.0
% STD DEV	8.2	8.8	26.4	8.8	6.1	0.5	0.0

TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (μM)	0.00	0.20	0.6	2.0	6.4	20.1	63.5
SAMPLE 1	17.0550	12.2300	12.4700	8.3400	5.5700	2.4840	1.9340
SAMPLE 2	15.8700	16.3600	13.9700	9.8200	5.9910	3.7490	2.8670
SAMPLE 3	12.7800	16.7800	14.7000	9.8300	6.3400	4.4800	3.0250
MEAN	15.2350	15.1233	13.7133	9.3300	5.9670	3.5710	2.6087
% CONTROL	100.0	99.3	90.0	61.2	39.2	23.4	17.1
% STD DEV	14.5	16.5	7.5	5.6	2.5	6.6	3.9

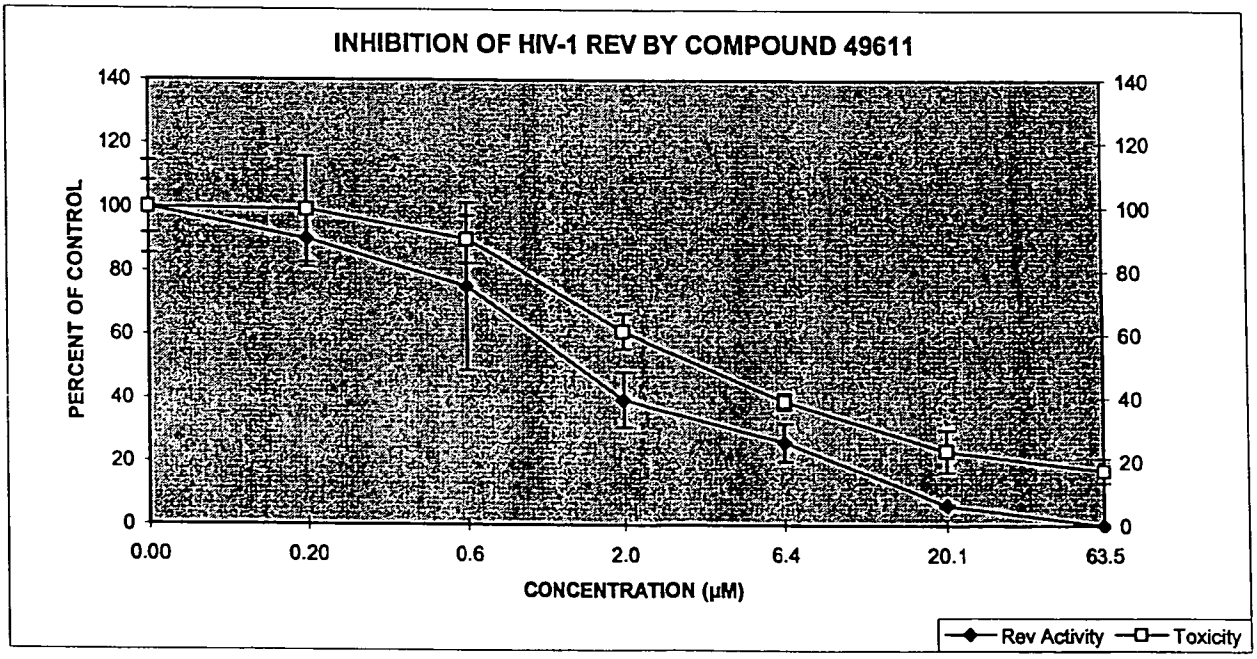
IC50 (μM) = 1.43
IC90 (μM) = 16.1

TC50 (μM) = 3.61
TC90 (μM) = >63.5

TI50 = 2.52
TI90 = >3.94

Dox Renilla Control = 0.0295
Dox Firefly Control = 2.5100

Renilla Background = 0.0003
Firefly Background = 0.0002



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Compound 73497

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (μM)	0.00	0.29	0.9	2.9	9.1	28.7	90.6
SAMPLE 1	0.1270	0.1665	0.1428	0.1422	0.0764	0.0299	0.0038
SAMPLE 2	0.1278	0.1412	0.1583	0.1158	0.0710	0.0303	0.0048
SAMPLE 3	0.1464	0.1983	0.1599	0.0896	0.0520	0.0332	0.0041
MEAN	0.1337	0.1687	0.1537	0.1159	0.0665	0.0312	0.0043
% CONTROL	100.0	126.1	114.9	86.7	49.7	23.3	3.2
% STD DEV	8.2	21.4	7.1	19.7	9.6	1.3	0.4

TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (μM)	0.00	0.29	0.9	2.9	9.1	28.7	90.6
SAMPLE 1	17.0550	17.7000	17.2100	17.0700	12.6400	8.1500	7.1740
SAMPLE 2	15.8700	17.3200	18.7900	16.4400	12.3300	8.0600	7.3930
SAMPLE 3	12.7800	17.4900	18.2800	16.1800	11.7100	8.2100	6.7830
MEAN	15.2350	17.5033	18.0933	16.5633	12.2267	8.1400	7.1167
% CONTROL	100.0	114.9	118.8	108.7	80.3	53.4	46.7
% STD DEV	14.5	1.2	5.3	3.0	3.1	0.5	2.0

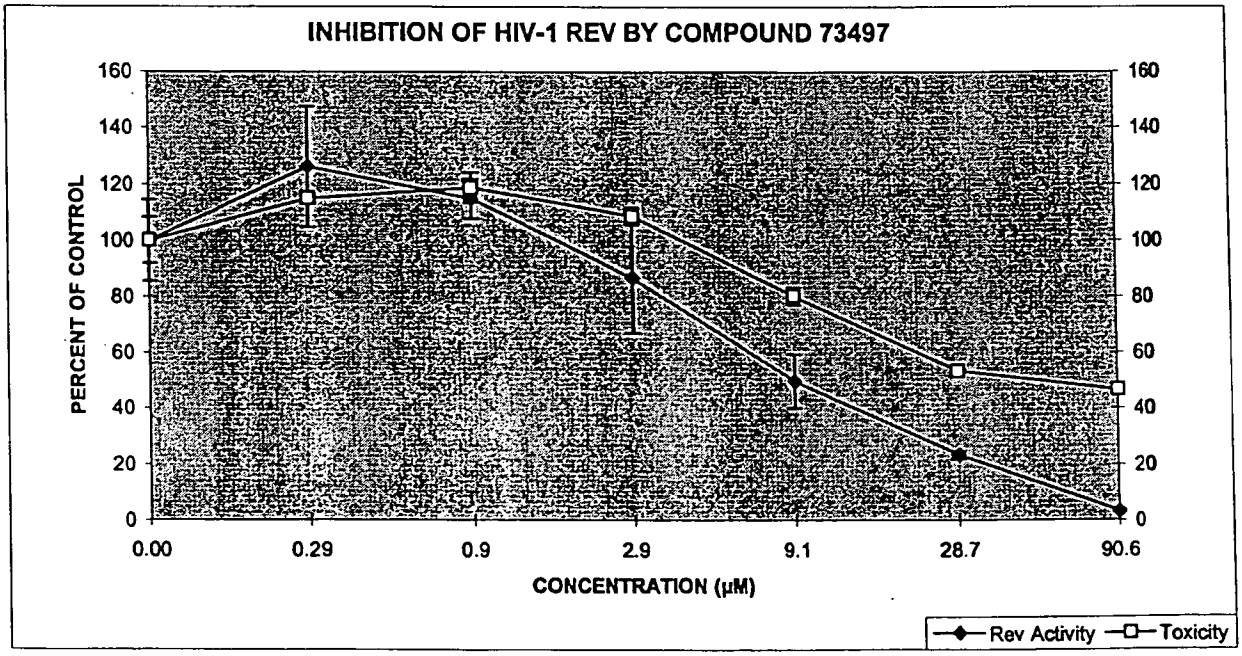
IC50 (μM) = 8.99
IC90 (μM) = 61.4

TC50 (μM) = 51.6
TC90 (μM) = >90.6

TI50 = 5.74
TI90 = >1.48

Dox Renilla Control = 0.0295
Dox Firefly Control = 2.5100

Renilla Background = 0.0003
Firefly Background = 0.0002



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Compound 74377

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (μM)	0.00	0.13	0.4	1.3	4.2	13.2	41.8
SAMPLE 1	0.1270	0.1255	0.1717	0.1323	0.0903	0.0613	0.0249
SAMPLE 2	0.1278	0.1706	0.1213	0.1207	0.1012	0.0702	0.0427
SAMPLE 3	0.1464	0.1547	0.1396	0.1623	0.1281	0.0495	0.0488
MEAN	0.1337	0.1503	0.1442	0.1385	0.1066	0.0604	0.0388
% CONTROL	100.0	112.4	107.9	103.5	79.7	45.1	29.0
% STD DEV	8.2	17.1	19.1	16.1	14.5	7.8	9.3

TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (μM)	0.00	0.13	0.4	1.3	4.2	13.2	41.8
SAMPLE 1	17.0550	18.6500	19.1400	16.8100	14.9300	10.5400	9.6000
SAMPLE 2	15.8700	17.3200	18.5500	17.5500	15.0500	11.1300	9.0400
SAMPLE 3	12.7800	17.7400	18.3300	16.6900	14.7500	10.6300	8.9200
MEAN	15.2350	17.9033	18.6733	17.0167	14.9100	10.7667	9.1867
% CONTROL	100.0	117.5	122.6	111.7	97.9	70.7	60.3
% STD DEV	14.5	4.5	2.7	3.1	1.0	2.1	2.4

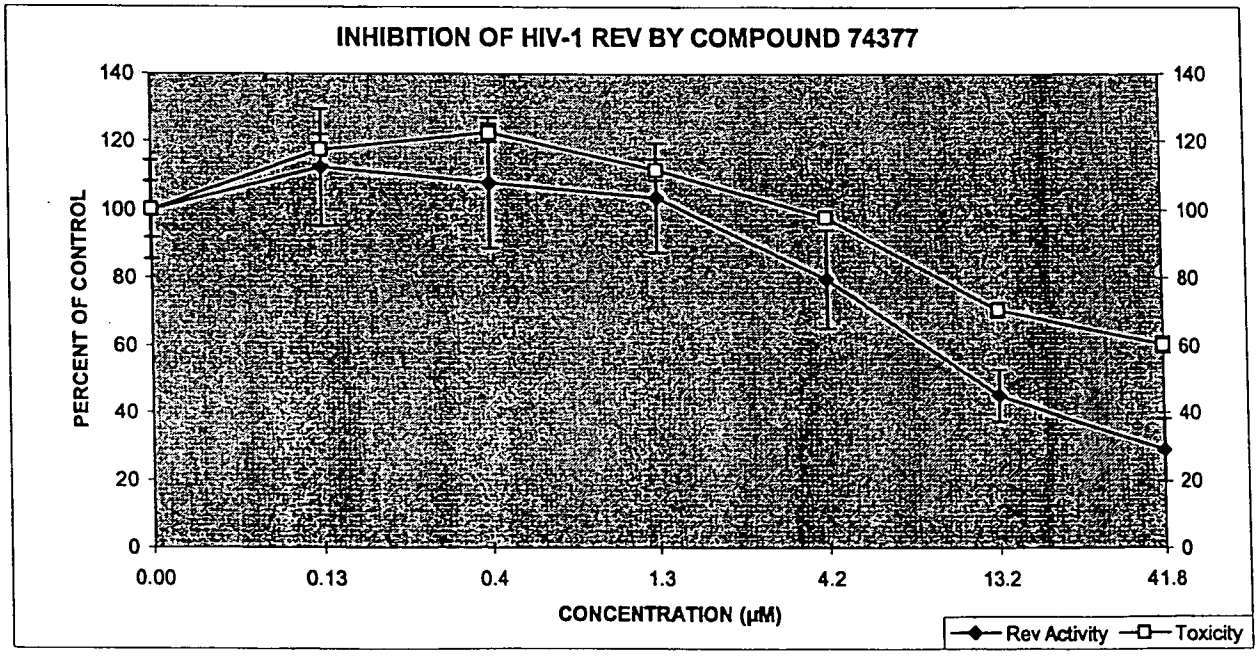
IC50 (μM) = 11.2
IC90 (μM) = >41.8

TC50 (μM) = >41.8
TC90 (μM) = >41.8

TI50 = >3.73
TI90 = N/A

Dox Renilla Control = 0.0295
Dox Firefly Control = 2.5100

Renilla Background = 0.0003
Firefly Background = 0.0002



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Compound 74378

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (μM)	0.00	0.14	0.4	1.4	4.3	13.7	43.3
SAMPLE 1	0.1270	0.1353	0.1485	0.1759	0.0998	0.0447	0.0548
SAMPLE 2	0.1278	0.1560	0.1556	0.1356	0.0968	0.0669	0.0357
SAMPLE 3	0.1464	0.1338	0.1398	0.1136	0.0978	0.0665	0.0268
MEAN	0.1337	0.1417	0.1480	0.1417	0.0982	0.0594	0.0391
% CONTROL	100.0	106.0	110.7	106.0	73.4	44.4	29.3
% STD DEV	8.2	9.3	5.9	23.6	1.1	9.5	10.7

TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (μM)	0.00	0.14	0.4	1.4	4.3	13.7	43.3
SAMPLE 1	17.0550	16.7000	16.4400	15.8800	13.8500	9.6100	7.6500
SAMPLE 2	15.8700	17.4600	15.8500	14.4800	13.9400	9.7800	7.6700
SAMPLE 3	12.7800	16.5100	14.7500	10.4700	12.7000	8.4600	5.9900
MEAN	15.2350	16.8900	15.6800	13.6100	13.4967	9.2833	7.1033
% CONTROL	100.0	110.9	102.9	89.3	88.6	60.9	46.6
% STD DEV	14.5	3.3	5.6	18.4	4.5	4.7	6.3

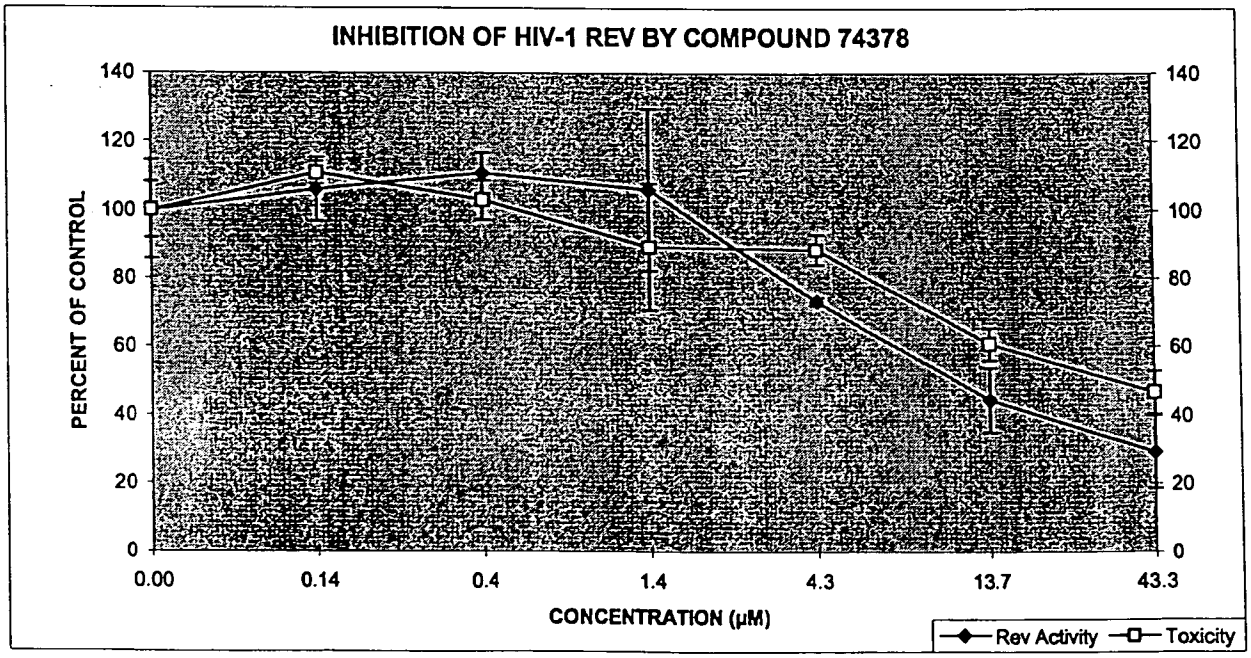
IC50 (μM) = 11.0
IC90 (μM) = >43.2

TC50 (μM) = 33.0
TC90 (μM) = >43.2

TI50 = 3.00
TI90 = N/A

Dox Renilla Control = 0.0295
Dox Firefly Control = 2.5100

Renilla Background = 0.0003
Firefly Background = 0.0002



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Compound 75168

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (µM)	0.00	0.08	0.3	0.8	2.7	8.5	26.8
SAMPLE 1	0.1140	0.1202	0.0981	0.0772	0.0502	0.0225	0.0000
SAMPLE 2	0.1219	0.1361	0.1336	0.1120	0.0486	0.0166	0.0000
SAMPLE 3	0.1300	0.1195	0.1310	0.1165	0.0723	0.0195	0.0000
MEAN	0.1220	0.1253	0.1209	0.1019	0.0571	0.0196	0.0000
%CONTROL	100.0	102.7	99.1	83.6	46.8	16.0	0.0
%STD DEV	6.6	7.7	16.2	17.6	10.9	2.4	0.0

TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (µM)	0.00	0.08	0.3	0.8	2.7	8.5	26.8
SAMPLE 1	14.6240	17.2690	15.8690	14.2690	10.7490	7.5320	5.9110
SAMPLE 2	14.6690	16.9490	17.6090	14.6490	11.0390	6.8760	4.6100
SAMPLE 3	14.4240	18.1990	17.8590	15.4290	10.4790	6.8060	5.3360
MEAN	14.5723	17.4723	17.1123	14.7823	10.7557	7.0713	5.2857
%CONTROL	100.0	119.9	117.4	101.4	73.8	48.5	36.3
%STD DEV	0.9	4.5	7.4	4.1	1.9	2.7	4.5

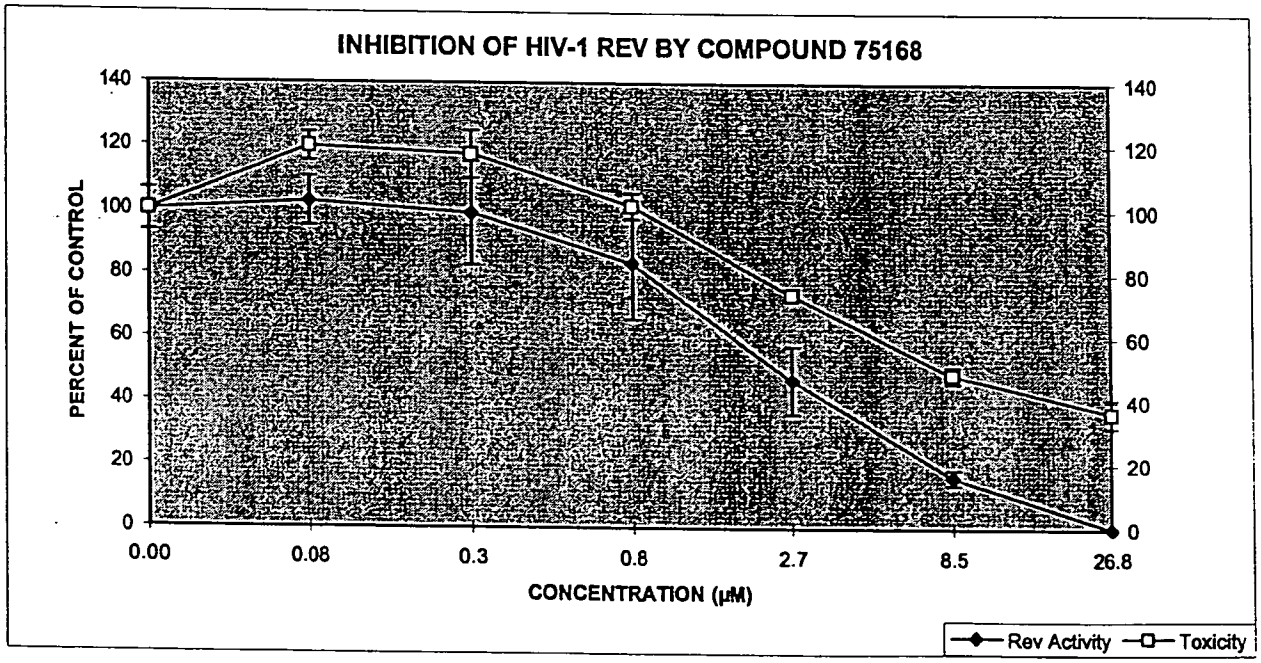
IC50 (µM) = 2.42
IC90 (µM) = 13.0

TC50 (µM) = 7.91
TC90 (µM) = >26.8

TI50 = 3.27
TI90 = >2.06

Dox Renilla Control = 0.0284
Dox Firefly Control = 2.4610

Renilla Background = 0.0003
Firefly Background = 0.0001



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Compound 89246

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (µM)	0.00	0.17	0.5	1.7	5.5	17.2	54.5
SAMPLE 1	0.1140	0.1060	0.0513	0.0178	0.0000	0.0000	0.0000
SAMPLE 2	0.1219	0.0911	0.0636	0.0298	0.0000	0.0000	0.0000
SAMPLE 3	0.1300	0.1106	0.0513	0.0183	0.0000	0.0000	0.0000
MEAN	0.1220	0.1026	0.0554	0.0220	0.0000	0.0000	0.0000
% CONTROL	100.0	84.1	45.4	18.0	0.0	0.0	0.0
% STD DEV	6.6	8.4	5.8	5.6	0.0	0.0	0.0

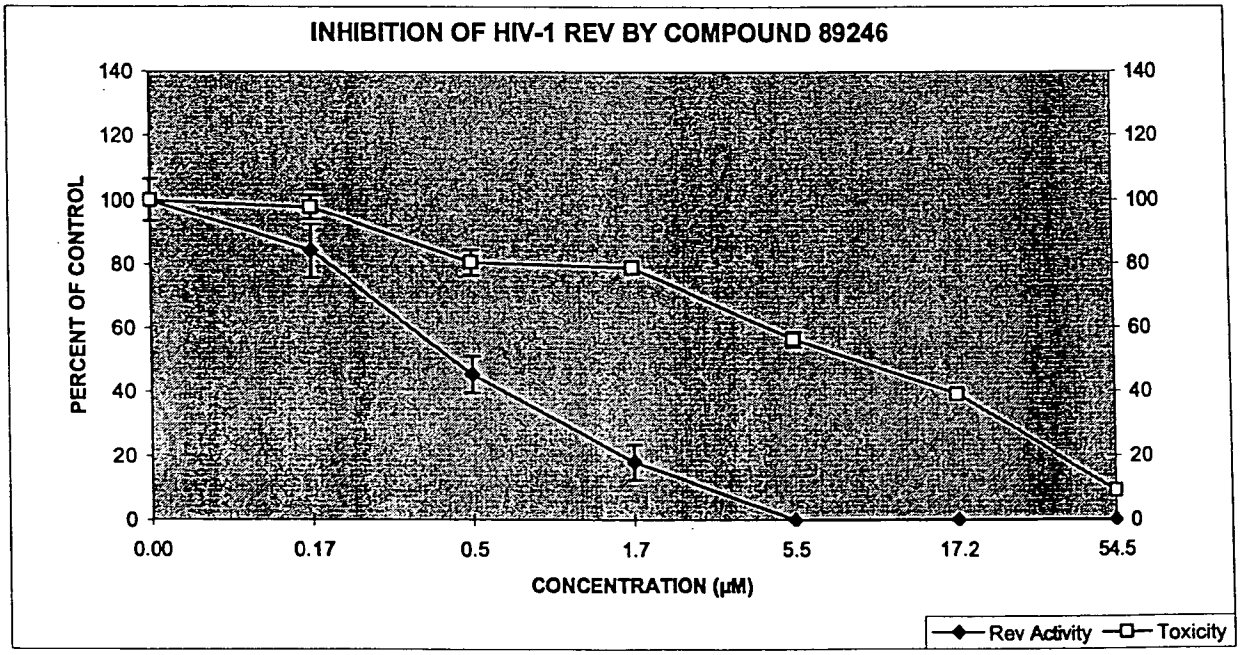
TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (µM)	0.00	0.17	0.5	1.7	5.5	17.2	54.5
SAMPLE 1	14.6240	14.7090	12.3290	11.8090	8.5890	5.8900	1.4300
SAMPLE 2	14.6690	13.6290	11.7390	11.4290	8.2390	5.7910	1.3820
SAMPLE 3	14.4240	14.4390	11.1490	11.2390	7.8690	5.4750	1.1120
MEAN	14.5723	14.2590	11.7390	11.4923	8.2323	5.7187	1.3080
% CONTROL	100.0	97.8	80.6	78.9	56.5	39.2	9.0
% STD DEV	0.9	3.9	4.0	2.0	2.5	1.5	1.2

IC50 (µM) = 0.48 | TC50 (µM) = 8.41 | TI50 = 17.52

IC90 (µM) = 2.88 | TC90 (µM) = 52.4 | TI90 = 18.19

Dox Renilla Control = 0.0284
Dox Firefly Control = 2.4610

Renilla Background = 0.0003
Firefly Background = 0.0001



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Compound 91161

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (μM)	0.00	0.12	0.4	1.2	4.0	12.5	39.5
SAMPLE 1	0.1140	0.1191	0.1498	0.1664	0.1668	0.0384	0.0000
SAMPLE 2	0.1219	0.1642	0.1256	0.1832	0.1378	0.0430	0.0000
SAMPLE 3	0.1300	0.1402	0.1752	0.1426	0.1375	0.0298	0.0000
MEAN	0.1220	0.1412	0.1502	0.1641	0.1474	0.0371	0.0000
% CONTROL	100.0	115.8	123.2	134.5	120.8	30.4	0.0
% STD DEV	6.6	18.5	20.3	16.7	13.8	5.5	0.0

TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (μM)	0.00	0.12	0.4	1.2	4.0	12.5	39.5
SAMPLE 1	14.6240	16.7690	16.9890	17.8990	17.1690	21.0890	7.6990
SAMPLE 2	14.6690	16.6990	16.0990	16.5690	16.2290	20.2890	7.0500
SAMPLE 3	14.4240	15.7190	15.6790	16.1190	16.1190	19.6990	7.7590
MEAN	14.5723	16.3957	16.2557	16.8623	16.5057	20.3590	7.5027
% CONTROL	100.0	112.5	111.6	115.7	113.3	139.7	51.5
% STD DEV	0.9	4.0	4.6	6.4	4.0	4.8	2.7

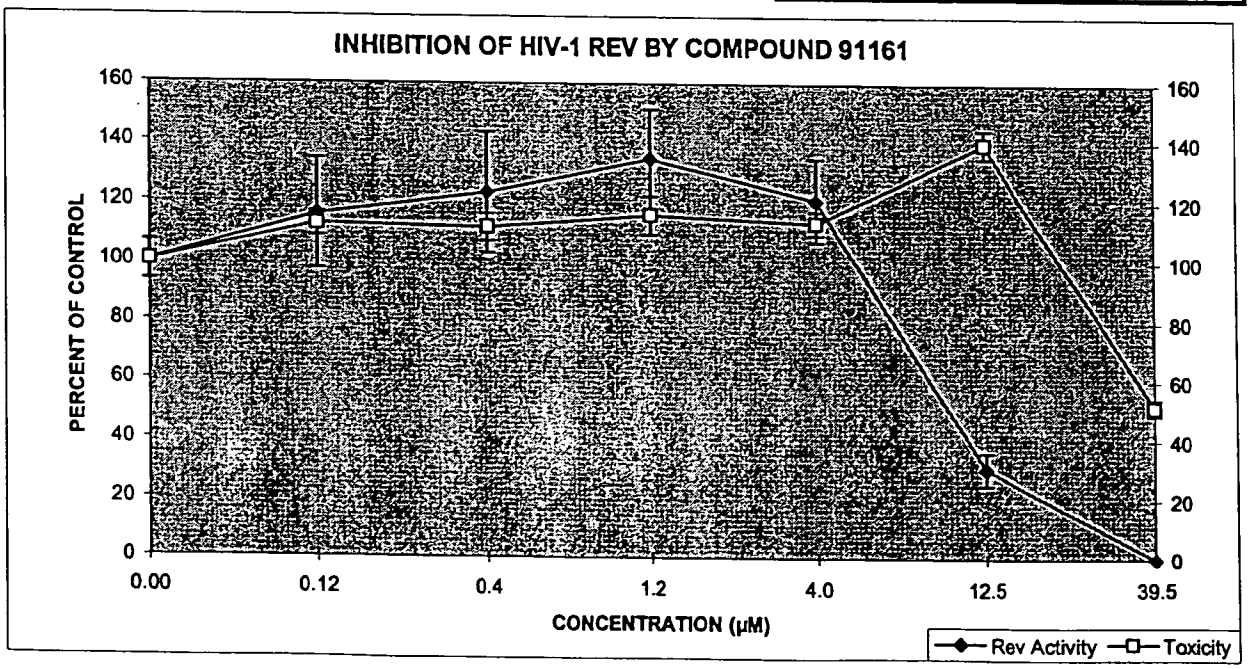
IC50 (μM) = 9.73
IC90 (μM) = 27.0

TC50 (μM) = >39.5
TC90 (μM) = >39.5

TI50 = ≥4.06
TI90 = ≥1.46

Dox Renilla Control = 0.0284
Dox Firefly Control = 2.4610

Renilla Background = 0.0003
Firefly Background = 0.0001



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Compound 103833

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (μM)	0.00	0.12	0.4	1.2	3.8	11.9	37.6
SAMPLE 1	0.1140	0.1570	0.1322	0.0554	0.0244	0.0016	0.0000
SAMPLE 2	0.1219	0.1290	0.1080	0.0602	0.0228	0.0035	0.0000
SAMPLE 3	0.1300	0.1262	0.0967	0.0518	0.0215	0.0004	0.0000
MEAN	0.1220	0.1374	0.1123	0.0558	0.0229	0.0019	0.0000
% CONTROL	100.0	112.7	92.1	45.8	18.8	1.5	0.0
% STD DEV	6.6	14.0	14.9	3.5	1.2	1.3	0.0

TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (μM)	0.00	0.12	0.4	1.2	3.8	11.9	37.6
SAMPLE 1	14.6240	13.8990	11.4690	10.1290	8.6890	7.9690	6.3870
SAMPLE 2	14.6690	13.2590	11.7290	9.9690	8.7790	7.6190	7.8590
SAMPLE 3	14.4240	12.8890	11.4090	9.4990	8.2090	8.0390	7.6190
MEAN	14.5723	13.3490	11.5357	9.8657	8.5590	7.8757	7.2883
% CONTROL	100.0	91.6	79.2	67.7	58.7	54.0	50.0
% STD DEV	0.9	3.5	1.2	2.2	2.1	1.5	5.4

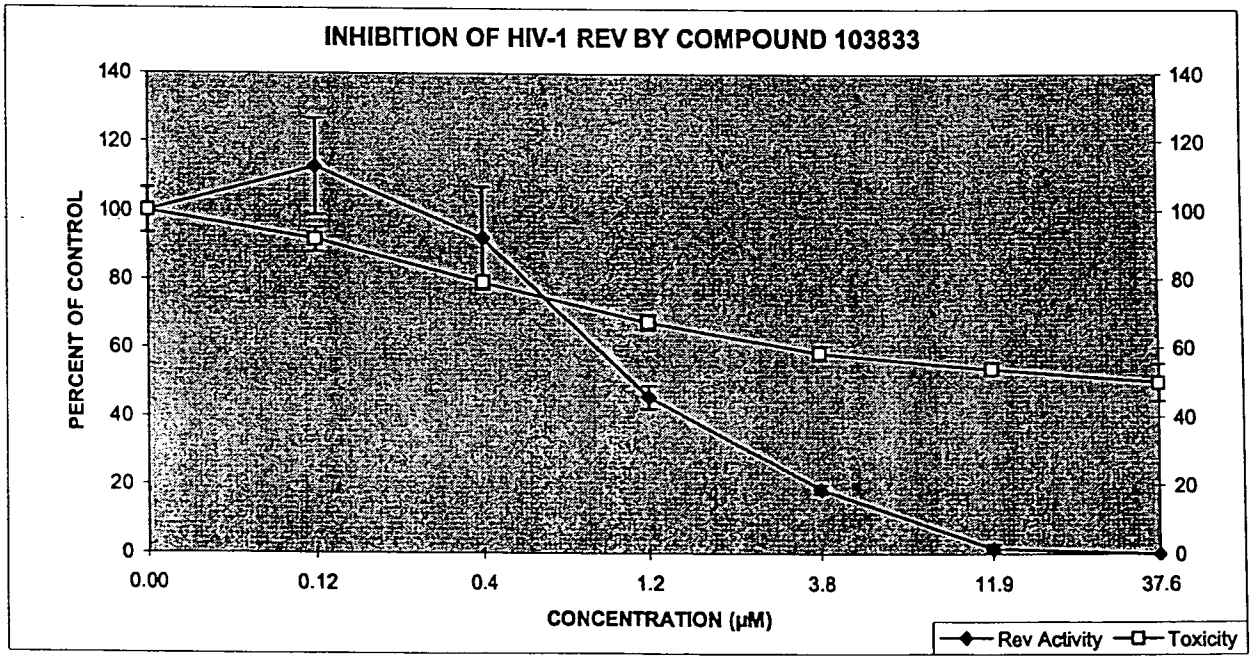
IC50 (μM) = 1.07
IC90 (μM) = 6.75

TC50 (μM) = >37.6
TC90 (μM) = >37.6

TI50 = >35.14
TI90 = >5.57

Dox Renilla Control = 0.0284
Dox Firefly Control = 2.4610

Renilla Background = 0.0003
Firefly Background = 0.0001



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Compound 104366

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)

CONC (μM)	0.00	0.25	0.8	2.5	7.9	25.1	79.5
SAMPLE 1	0.1452	0.1004	0.0973	0.0516	0.0059	0.0000	0.0000
SAMPLE 2	0.1398	0.1347	0.1403	0.0811	0.0190	0.0000	0.0000
SAMPLE 3	0.1650	0.1450	0.1126	0.0785	0.0147	0.0000	0.0000
MEAN	0.1500	0.1267	0.1167	0.0704	0.0132	0.0000	0.0000
% CONTROL	100.0	84.5	77.8	46.9	8.8	0.0	0.0
% STD DEV	8.8	15.6	14.5	10.9	4.5	0.0	0.0

TOXICITY: Firefly Luciferase Values (relative light units)

CONC (μM)	0.00	0.25	0.8	2.5	7.9	25.1	79.5
SAMPLE 1	14.7155	15.0805	15.4105	13.1405	12.4105	5.0215	0.0000
SAMPLE 2	15.4605	17.0805	15.1605	14.4505	12.9905	4.8665	0.0000
SAMPLE 3	14.9255	17.7505	16.3305	14.1805	12.3605	5.2375	0.0000
MEAN	15.0338	16.6372	15.6338	13.9238	12.5872	5.0418	0.0000
% CONTROL	100.0	110.7	104.0	92.6	83.7	33.5	0.0
% STD DEV	2.6	9.2	4.1	4.6	2.3	1.2	0.0

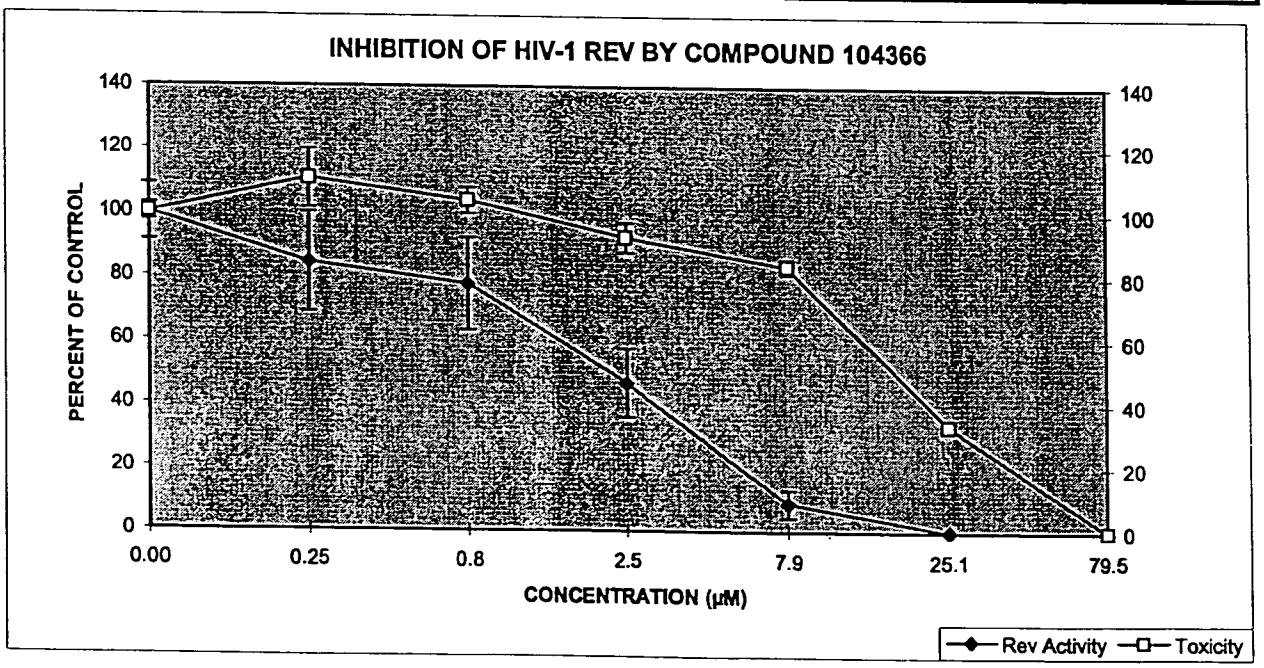
IC50 (μM) = 2.24
IC90 (μM) = 7.67

TC50 (μM) = 17.2
TC90 (μM) = 56.4

TI50 = 7.68
TI90 = 7.35

Dox Renilla Control = 0.0255
Dox Firefly Control = 2.1195

Renilla Background = 0.0004
Firefly Background = 0.0006



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Compound 107129

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)

CONC (μM)	0.00	0.17	0.5	1.7	5.3	16.8	53.0
SAMPLE 1	0.1452	0.1917	0.1264	0.1527	0.1017	0.0454	0.0000
SAMPLE 2	0.1398	0.1151	0.1294	0.1384	0.0961	0.0193	0.0000
SAMPLE 3	0.1650	0.1607	0.1318	0.1558	0.0877	0.0184	0.0000
MEAN	0.1500	0.1558	0.1292	0.1490	0.0952	0.0277	0.0000
% CONTROL	100.0	103.9	86.1	99.3	63.5	18.5	0.0
% STD DEV	8.8	25.7	1.8	6.2	4.7	10.2	0.0

TOXICITY: Firefly Luciferase Values (relative light units)

CONC (μM)	0.00	0.17	0.5	1.7	5.3	16.8	53.0
SAMPLE 1	14.7155	18.0605	15.3305	17.2605	19.5005	17.1105	0.0000
SAMPLE 2	15.4605	15.5205	16.4605	17.2705	17.9805	15.6405	0.0000
SAMPLE 3	14.9255	17.1105	16.2105	17.4505	18.2105	15.2705	0.0000
MEAN	15.0338	16.8972	16.0005	17.3272	18.5638	16.0072	0.0000
% CONTROL	100.0	112.4	106.4	115.3	123.5	106.5	0.0
% STD DEV	2.6	8.5	3.9	0.7	5.4	6.5	0.0

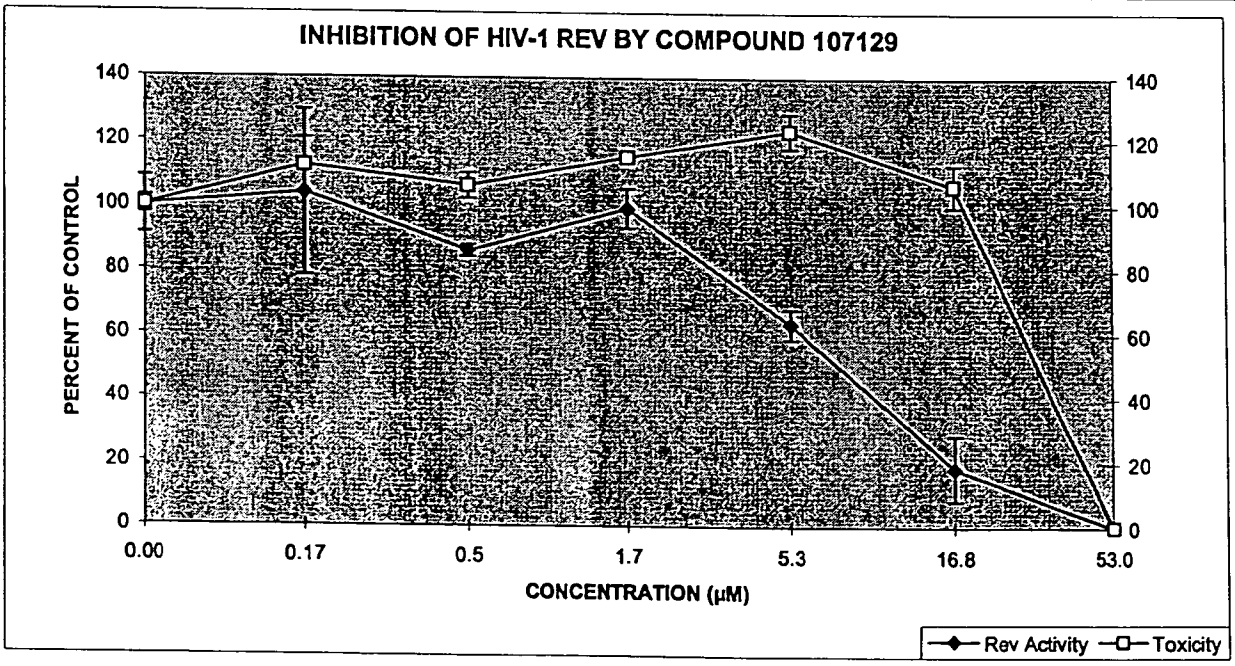
IC50 (μM) = 7.48
IC90 (μM) = 28.4

TC50 (μM) = 30.9
TC90 (μM) = 47.6

TI50 = 4.13
TI90 = 1.68

Dox Renilla Control = 0.0255
Dox Firefly Control = 2.1195

Renilla Background = 0.0004
Firefly Background = 0.0006



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Compound 107740

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (μM)	0.00	0.09	0.3	0.9	2.9	9.2	29.3
SAMPLE 1	0.1392	0.1477	0.1956	0.1849	0.1485	0.0571	0.0000
SAMPLE 2	0.1280	0.1290	0.1582	0.1421	0.1121	0.0478	0.0000
SAMPLE 3	0.1301	0.0999	0.1478	0.1717	0.1579	0.0620	0.0000
MEAN	0.1324	0.1256	0.1672	0.1663	0.1395	0.0557	0.0000
%CONTROL	100.0	94.8	126.3	125.6	105.4	42.0	0.0
%STD DEV	4.5	18.2	19.0	16.6	18.3	5.4	0.0

TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (μM)	0.00	0.09	0.3	0.9	2.9	9.2	29.3
SAMPLE 1	14.1198	10.6248	14.9548	15.4148	15.0048	12.2348	3.6738
SAMPLE 2	14.5348	16.8848	18.2748	18.2548	16.9148	12.1048	4.1198
SAMPLE 3	14.3948	16.2848	16.5248	17.9148	17.5348	12.1548	3.9238
MEAN	14.3498	14.5982	16.5848	17.1948	16.4848	12.1648	3.9058
%CONTROL	100.0	101.7	115.6	119.8	114.9	84.8	27.2
%STD DEV	1.5	24.1	11.6	10.8	9.2	0.5	1.6

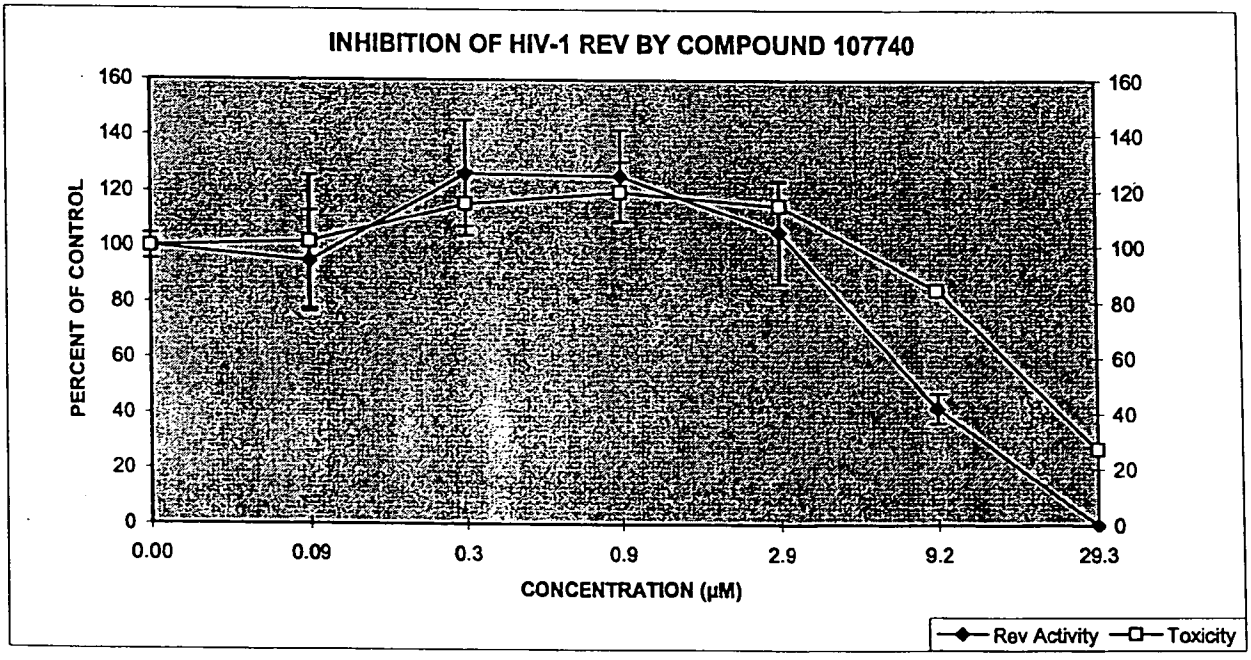
IC50 (μM) = 8.00
IC90 (μM) = 22.2

TC50 (μM) = 18.5
TC90 (μM) = >29.2

TI50 = 2.31
TI90 = >1.32

Dox Renilla Control = 0.0303
Dox Firefly Control = 2.0152

Renilla Background = 0.0004
Firefly Background = 0.0008



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Compound 109020

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (μM)	0.00	0.13	0.4	1.3	4.1	12.9	40.8
SAMPLE 1	0.1392	0.1049	0.1391	0.1295	0.1050	0.0504	0.0000
SAMPLE 2	0.1280	0.1471	0.1397	0.1221	0.0778	0.0469	0.0085
SAMPLE 3	0.1301	0.1242	0.1421	0.1347	0.1055	0.0603	0.0025
MEAN	0.1324	0.1254	0.1403	0.1288	0.0961	0.0526	0.0037
% CONTROL	100.0	94.7	106.0	97.3	72.6	39.7	2.8
% STD DEV	4.5	16.0	1.2	4.8	12.0	5.2	3.3

TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (μM)	0.00	0.13	0.4	1.3	4.1	12.9	40.8
SAMPLE 1	14.1198	16.7448	17.7248	18.1848	19.2348	20.9648	18.0148
SAMPLE 2	14.5348	14.2148	16.0648	17.1448	18.6248	21.2948	18.4048
SAMPLE 3	14.3948	16.2648	17.0048	17.5648	19.8748	21.2948	18.4148
MEAN	14.3498	15.7415	16.9315	17.6315	19.2448	21.1848	18.2782
% CONTROL	100.0	109.7	118.0	122.9	134.1	147.6	127.4
% STD DEV	1.5	9.4	5.8	3.6	4.4	1.3	1.6

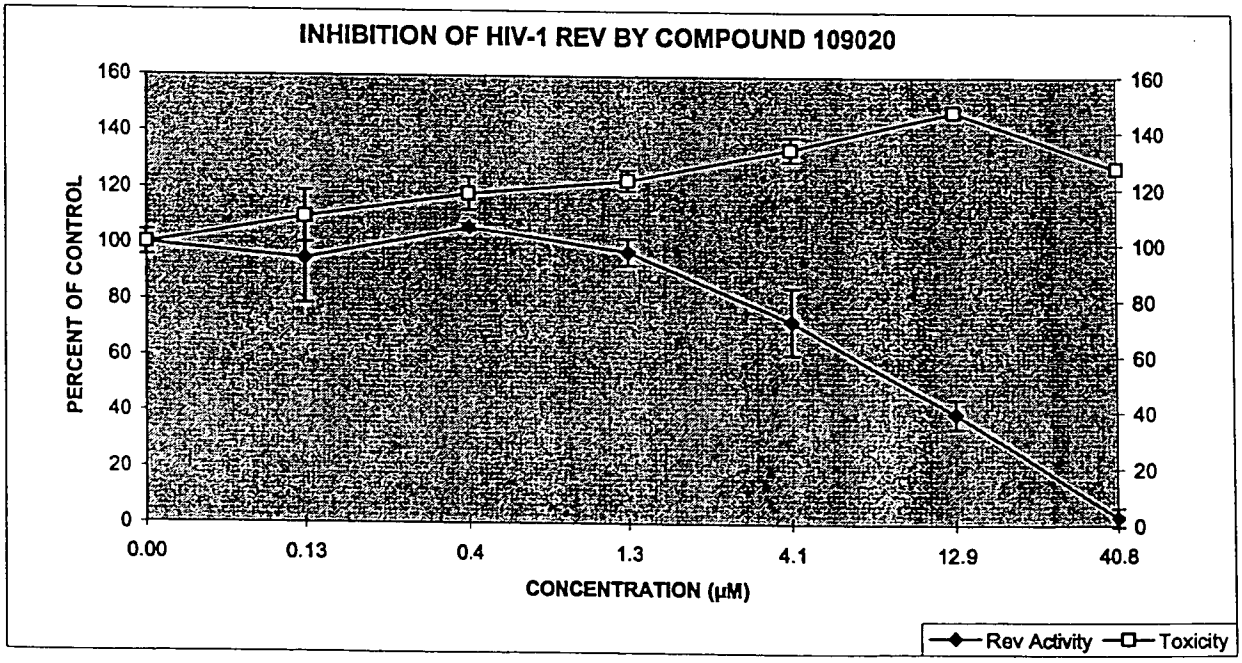
IC50 (μM) = 8.98
IC90 (μM) = 32.5

TC50 (μM) = >40.8
TC90 (μM) = >40.8

TI50 = >4.54
TI90 = >1.26

Dox Renilla Control = 0.0303
Dox Firefly Control = 2.0152

Renilla Background = 0.0004
Firefly Background = 0.0008



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Leptomycin B

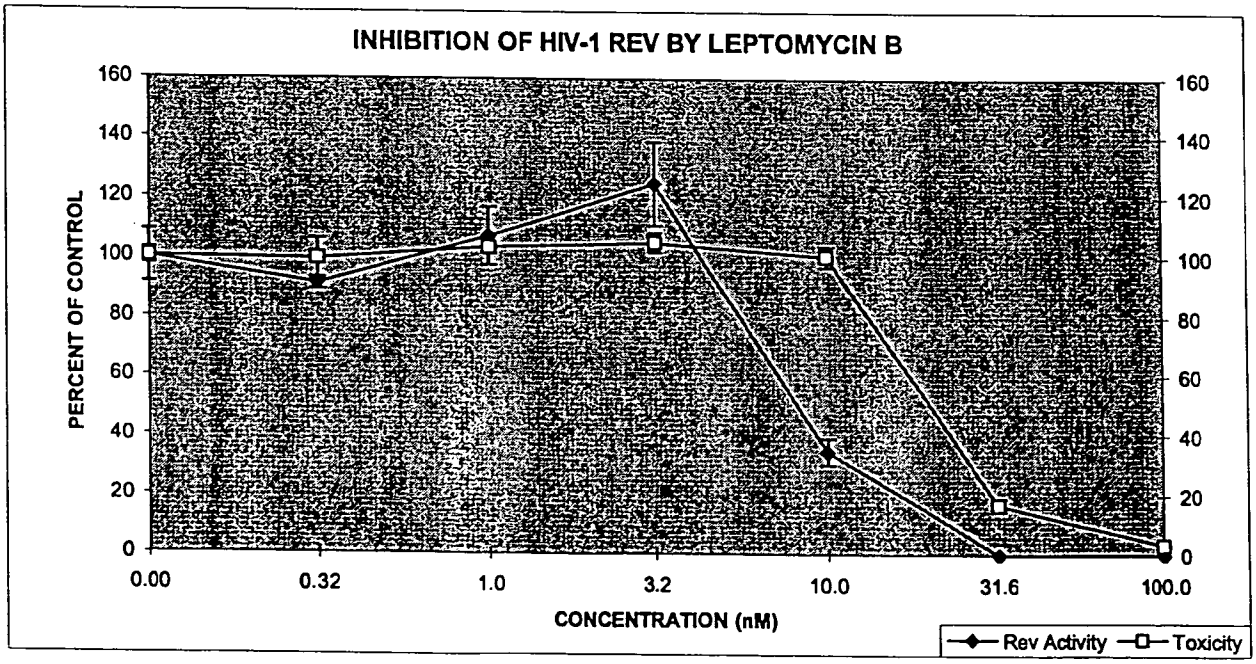
ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (nM)	0.00	0.32	1.0	3.2	10.0	31.6	100.0
SAMPLE 1	0.1452	0.1365	0.1476	0.1637	0.0539	0.0000	0.0000
SAMPLE 2	0.1398	0.1336	0.1764	0.2041	0.0445	0.0000	0.0000
SAMPLE 3	0.1650	0.1399	0.1574	0.1933	0.0577	0.0000	0.0000
MEAN	0.1500	0.1367	0.1605	0.1870	0.0520	0.0000	0.0000
% CONTROL	100.0	91.1	107.0	124.7	34.7	0.0	0.0
% STD DEV	8.8	2.1	9.8	13.9	4.5	0.0	0.0

TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (nM)	0.00	0.32	1.0	3.2	10.0	31.6	100.0
SAMPLE 1	14.7155	15.5305	15.8305	16.3305	15.0005	2.6545	0.4875
SAMPLE 2	15.4605	15.5305	15.4205	15.4805	15.6705	2.7455	0.5335
SAMPLE 3	14.9255	13.7705	15.2905	15.5305	14.6205	2.1725	0.4735
MEAN	15.0338	14.9438	15.5138	15.7805	15.0972	2.5242	0.4982
% CONTROL	100.0	99.4	103.2	105.0	100.4	16.8	3.3
% STD DEV	2.6	6.8	1.9	3.2	3.5	2.0	0.2

IC50 (nM) = 8.22	TC50 (nM) = 20.0	TI50 = 2.43
IC90 (nM) = 22.7	TC90 (nM) = 56.5	TI90 = 2.49

Dox. Renilla Control = 0.0255
Dox. Firefly Control = 2.1195

Renilla Background = 0.0004
Firefly Background = 0.0006



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Leptomycin B

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (nM)	0.00	0.32	1.0	3.2	10.0	31.6	100.0
SAMPLE 1	0.1452	0.1345	0.1531	0.1800	0.0644	0.0004	0.0000
SAMPLE 2	0.1398	0.1368	0.1433	0.1603	0.0403	0.0000	0.0000
SAMPLE 3	0.1650	0.1627	0.1415	0.1832	0.0565	0.0000	0.0000
MEAN	0.1500	0.1447	0.1460	0.1745	0.0537	0.0001	0.0000
% CONTROL	100.0	96.5	97.3	116.3	35.8	0.1	0.0
% STD DEV	8.8	10.4	4.2	8.3	8.2	0.2	0.0

TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (nM)	0.00	0.32	1.0	3.2	10.0	31.6	100.0
SAMPLE 1	14.7155	14.0305	15.0105	15.3005	14.2305	2.9095	0.4055
SAMPLE 2	15.4605	13.7705	14.8205	13.0905	12.3305	3.6635	0.6475
SAMPLE 3	14.9255	11.1805	13.9505	14.0605	12.6805	3.2605	0.5905
MEAN	15.0338	12.9938	14.5938	14.1505	13.0805	3.2778	0.5478
% CONTROL	100.0	86.4	97.1	94.1	87.0	21.8	3.6
% STD DEV	2.6	10.5	3.8	7.4	6.7	2.5	0.8

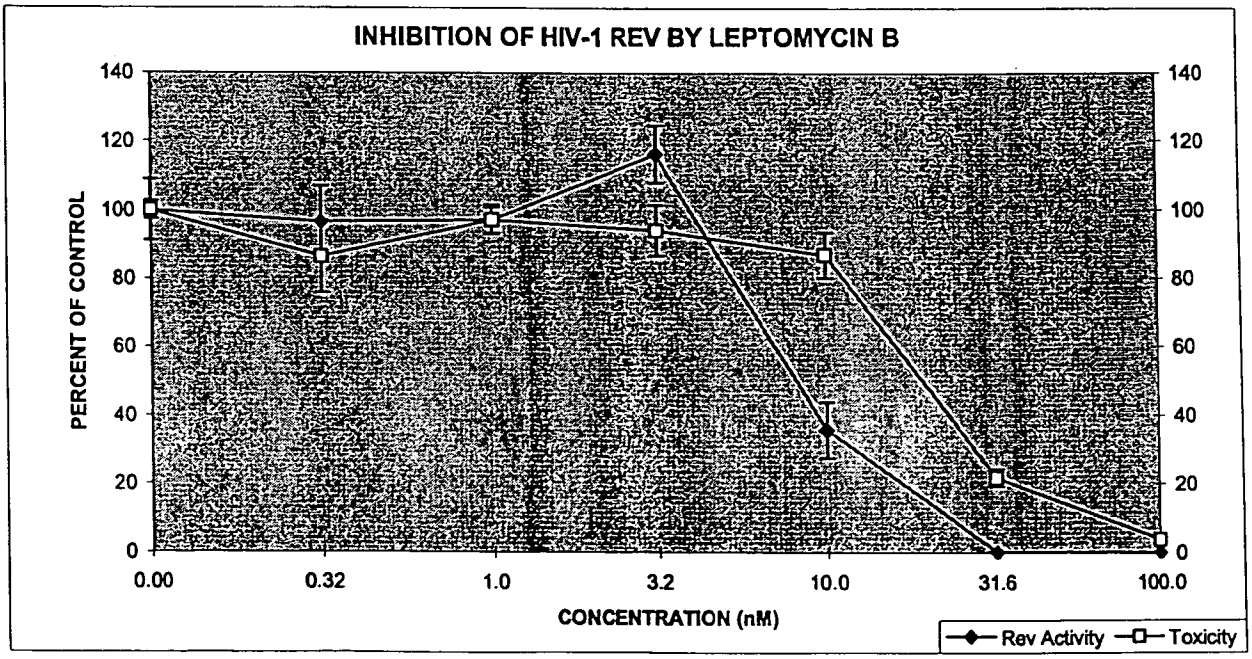
IC50 (nM) = 8.17
IC90 (nM) = 23.0

TC50 (nM) = 19.2
TC90 (nM) = 66.8

TI50 = 2.35
TI90 = 2.90

Dox Renilla Control = 0.0255
Dox Firefly Control = 21.195

Renilla Background = 0.0004
Firefly Background = 0.0006



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Leptomycin B

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (nM)	0.00	0.32	1.0	3.2	10.0	31.6	100.0
SAMPLE 1	0.1392	0.1237	0.2036	0.2014	0.0289	0.0000	0.0000
SAMPLE 2	0.1280	0.1333	0.1558	0.1592	0.0442	0.0000	0.0000
SAMPLE 3	0.1301	0.1202	0.1685	0.1638	0.0335	0.0000	0.0000
MEAN	0.1324	0.1258	0.1760	0.1748	0.0356	0.0000	0.0000
% CONTROL	100.0	95.0	132.9	132.0	26.8	0.0	0.0
% STD DEV	4.5	5.1	18.7	17.5	5.9	0.0	0.0

TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (nM)	0.00	0.32	1.0	3.2	10.0	31.6	100.0
SAMPLE 1	14.1198	15.3348	16.9848	17.7048	15.5848	3.2068	0.6368
SAMPLE 2	14.5348	15.4748	17.4348	17.3248	15.4348	2.6648	0.4548
SAMPLE 3	14.3948	15.0548	16.9448	16.1448	15.6948	2.6478	0.6728
MEAN	14.3498	15.2882	17.1215	17.0582	15.5715	2.8398	0.5882
% CONTROL	100.0	106.5	119.3	118.9	108.5	19.8	4.1
% STD DEV	1.5	1.5	1.9	5.7	0.9	2.2	0.8

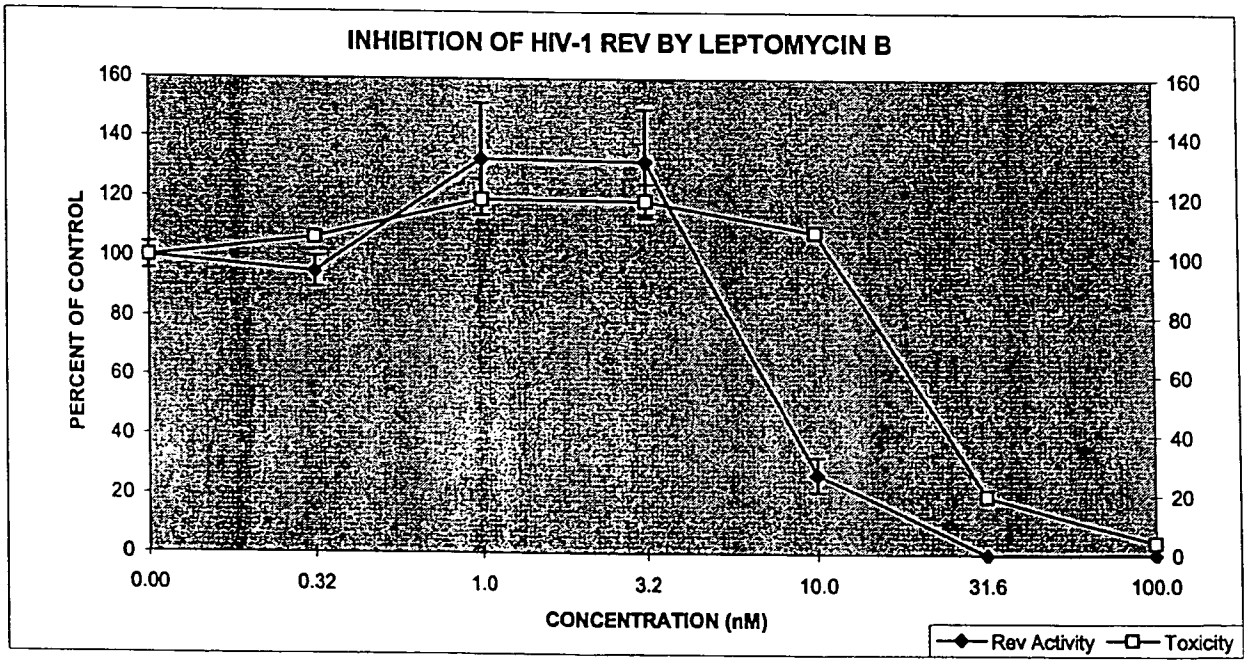
IC50 (nM) = 7.76
IC90 (nM) = 20.6

TC50 (nM) = 21.4
TC90 (nM) = 64.9

TI50 = 2.76
TI90 = 3.15

Dox Renilla Control = 0.0303
Dox Firefly Control = 2.0152

Renilla Background = 0.0004
Firefly Background = 0.0008



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

INHIBITION OF HIV-1 REV FUNCTION IN DLREV CELLS BY Leptomycin B

ANTI-REV ACTIVITY: Renilla Luciferase Values (relative light units)							
CONC (nM)	0.00	0.32	1.0	3.2	10.0	31.6	100.0
SAMPLE 1	0.1392	0.1300	0.1854	0.1848	0.0460	0.0000	0.0000
SAMPLE 2	0.1280	0.1432	0.1675	0.1474	0.0437	0.0000	0.0000
SAMPLE 3	0.1301	0.1794	0.1781	0.1294	0.0224	0.0000	0.0000
MEAN	0.1324	0.1509	0.1770	0.1539	0.0374	0.0000	0.0000
% CONTROL	100.0	113.9	133.7	116.2	28.2	0.0	0.0
% STD DEV	4.5	19.3	6.8	21.3	9.8	0.0	0.0

TOXICITY: Firefly Luciferase Values (relative light units)							
CONC (nM)	0.00	0.32	1.0	3.2	10.0	31.6	100.0
SAMPLE 1	14.1198	14.9748	16.3648	17.0948	15.5248	2.8948	0.4688
SAMPLE 2	14.5348	15.1348	16.4948	16.0648	14.2248	3.0558	0.5468
SAMPLE 3	14.3948	16.2048	16.7548	14.1348	14.0648	3.0888	0.7958
MEAN	14.3498	15.4382	16.5382	15.7648	14.6048	3.0132	0.6038
% CONTROL	100.0	107.6	115.2	109.9	101.8	21.0	4.2
% STD DEV	1.5	4.7	1.4	10.5	5.6	0.7	1.2

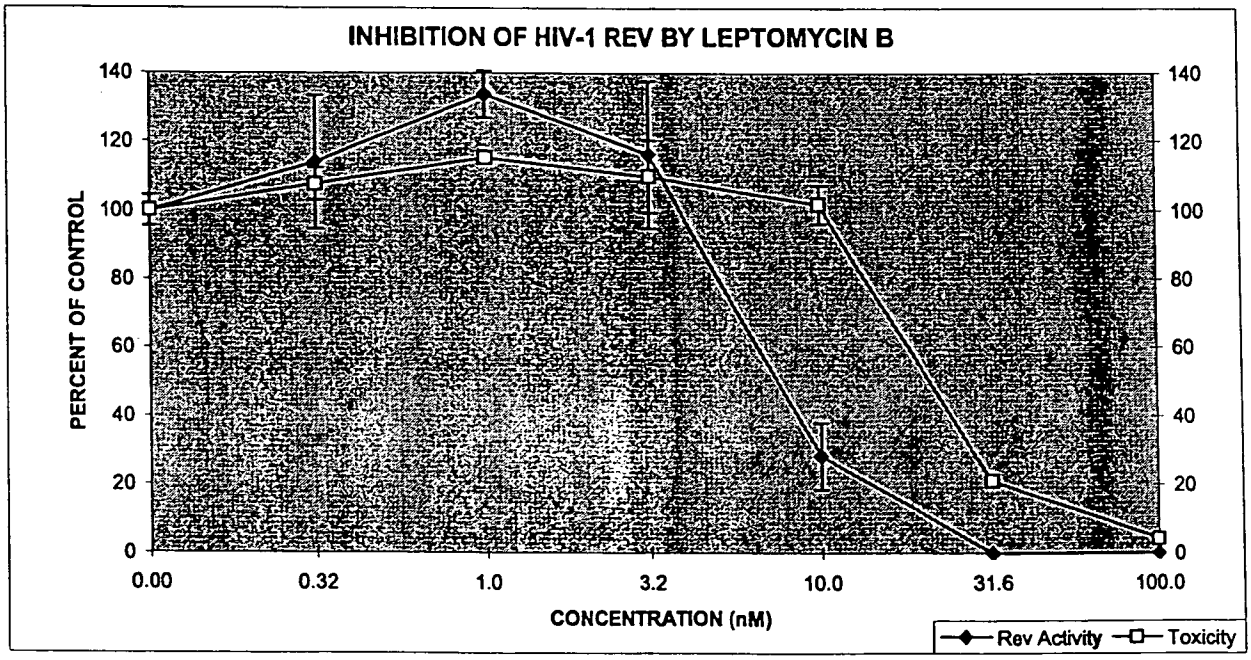
IC50 (nM) = 7.52
IC90 (nM) = 21.0

TC50 (nM) = 20.9
TC90 (nM) = 67.2

TI50 = 2.78
TI90 = 3.20

Dox Renilla Control = 0.0303
Dox Firefly Control = 2.0152

Renilla Background = 0.0004
Firefly Background = 0.0008



Client: Rekosh/Hammarskjöld
Contract: IR&D
Project: 1085

HIV-1 Rev Assay
Southern Research Institute

Setup Date: 03/17/04
Assay Date: 03/18/04
Technician: P. Ward

Anti-HIV Efficacy Evaluation in Fresh Human PBMCs

Fresh human PBMCs, seronegative for HIV and HBV, were isolated from screened donors (Interstate Blood Bank, Inc. Memphis, TN). Cells were pelleted/washed 2-3 times by low speed centrifugation and re-suspension in PBS to remove contaminating platelets. The Leukophoresed blood was then diluted 1:1 with Dulbecco's Phosphate Buffered Saline (DPBS) and layered over 14 mL of Lymphocyte Separation Medium (LSM; Cellgro® by Mediatech, Inc.; density 1.078+/-0.002 g/ml; Cat.# 85-072-CL) in a 50 mL centrifuge tube and then centrifuged for 30 minutes at 600 X g. Banded PBMCs were gently aspirated from the resulting interface and subsequently washed 2X with PBS by low speed centrifugation. After the final wash, cells were enumerated by trypan blue exclusion and re-suspended at 1 x 10^7 cells/mL in RPMI 1640 supplemented with 15 % Fetal Bovine Serum (FBS), and 2 mM L-glutamine, 4 µg/mL Phytohemagglutinin (PHA-P, Sigma). The cells were allowed to incubate for 48-72 hours at 37°C. After incubation, PBMCs were centrifuged and resuspended in RPMI 1640 with 15% FBS, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, 10 µg/mL gentamycin, and 20 U/mL recombinant human IL-2 (R&D Systems, Inc). IL-2 is included in the culture medium to maintain the cell division initiated by the PHA mitogenic stimulation. PBMCs were maintained in this medium at a concentration of 1-2 x 10^6 cells/mL with biweekly medium changes until used in the assay protocol. Cells were kept in culture for a maximum of two weeks before being deemed too old for use in assays and discarded. Monocytes were depleted from the culture as the result of adherence to the tissue culture flask.

For the standard PBMC assay, PHA-P stimulated cells from at least two normal donors were pooled (mixed together), diluted in fresh medium to a final concentration of 1 x 10^6 cells/mL, and plated in the interior wells of a 96 well round bottom microplate at 50 µL/well (5 x 10^4 cells/well) in a standard format developed by the Infectious Disease Research department of Southern Research Institute. Pooling (mixing) of mononuclear cells from more than one donor is used to minimize the variability observed between individual donors, which results from quantitative and qualitative differences in HIV infection and overall response to the PHA and IL-2 of primary lymphocyte populations. Each plate contains virus/cell control wells (cells plus virus), experimental wells (drug plus cells plus virus) and compound control wells (drug plus media without cells, necessary for MTS monitoring of cytotoxicity). Since HIV-1 is not cytopathic to PBMCs, this allows the use of the same assay plate for both antiviral activity and cytotoxicity measurements. Test drug dilutions were prepared at a 2X concentration in microtiter tubes and 100 µL of each concentration was placed in appropriate wells using the standard format. 50 µL of a predetermined dilution of virus stock was placed in each test well (final MOI ≅ 0.1). The PBMC cultures were maintained for seven days following infection at 37°C, 5% CO_2. After this period, cell-free supernatant samples were collected for analysis of reverse transcriptase activity or p24 antigen. Following removal of supernatant samples, compound cytotoxicity was measured by addition of MTS to the plates for determination of cell viability. Wells were also examined microscopically and any abnormalities were noted.

Reverse transcriptase activity assay

A microtiter plate-based reverse transcriptase (RT) reaction was utilized (Buckheit et al., AIDS Research and Human Retroviruses 7:295-302, 1991). Tritiated thymidine triphosphate (^{3}H-TTP, 80 Ci/mmol, NEN) was received in 1:1 dH_2O:Ethanol at 1 mCi/mL. Poly rA:oligo dT

template:primer (Pharmacia) was prepared as a stock solution by combining 150 µL poly rA (20 mg/mL) with 0.5 mL oligo dT (20 units/mL) and 5.35 mL sterile dH_2O followed by aliquoting (1.0 mL) and storage at -20°C. The RT reaction buffer was prepared fresh on a daily basis and consisted of 125 µL 1.0 M EGTA, 125 µL dH_2O, 125 µL 20% Triton X100, 50 µL 1.0 M Tris (pH 7.4), 50 µL 1.0 M DTT, and 40 µL 1.0 M $MgCl_2$. The final reaction mixture was prepared by combining 1 part ^{3}H-TTP, 4 parts dH_2O, 2.5 parts poly rA:oligo dT stock and 2.5 parts reaction buffer. Ten microliters of this reaction mixture was placed in a round bottom microtiter plate and 15 µL of virus containing supernatant was added and mixed. The plate was incubated at 37°C for 60 minutes. Following incubation, the reaction volume was spotted onto DE81 filter-mats (Wallac), washed 5 times for 5 minutes each in a 5% sodium phosphate buffer or 2X SSC (Life Technologies). Next they were washed 2 times for 1 minute each in distilled water, 2 times for 1 minute each in 70% ethanol, and then dried. Incorporated radioactivity (counts per minute, CPM) was quantified using standard liquid scintillation techniques.

MTS staining for PBMC viability to measure cytotoxicity

At assay termination, assay plates were stained with the soluble tetrazolium-based dye MTS (CellTiter 96 Reagent, Promega) to determine cell viability and quantify compound toxicity. The mitochondrial enzymes of metabolically active cells metabolize MTS to yield a soluble formazan product. This allows the rapid quantitative analysis of cell viability and compound cytotoxicity. The MTS is a stable solution that does not require preparation before use. At termination of the assay, 20 µL of MTS reagent was added per well. The microtiter plates were then incubated 4-6 hrs at 37°C. The incubation intervals were chosen based on empirically determined times for optimal dye reduction. Adhesive plate sealers were used in place of the lids, the sealed plate was inverted several times to mix the soluble formazan product and the plate was read spectrophotometrically at 490/650 nm with a Molecular Devices Vmax or SpectraMaxPlus plate reader.

Virus Information

The low-passage, lymphotropic clinical isolate HIV-1_{ROJO} was obtained from a pediatric patient attending the AIDS Clinic at the University of Alabama at Birmingham and was isolated in the laboratories of Southern Research Institute as previously described (Buckheit, et al. AIDS Res Hum Retroviruses. 1994 10:1497-506). HIV-1_{ROJO} has been typed as syncytium inducing (SI) in MT-2 cells and is presumed to be a subtype B (X4) isolate. The HIV-1 clinical isolate BR/93/021 was obtained from the NIH AIDS Research and Reference Reagent Program. This virus was originally isolated from a seropositive individual in Brazil and has been characterized as an Envelope Subtype B and as an R5-tropic isolate. The HIV-1 molecular clone NL4-3 was obtained from the NIH AIDS Research and Reference Reagent Program. Virus stocks were prepared by transfection of plasmid DNA into cells using standard techniques. A Pre-titered aliquot of each virus was removed from the freezer (LN_2) and thawed rapidly to room temperature in a biological safety cabinet immediately before use.

Compound Information

Compound	Stock Concentration Provided (mM)	High-Test Concentration Used (µM)
49611	25.4	63.5
73497	36.25	90.625
74377	16.7	41.75
74378	17.3	43.25
75168	10.7	26.75
89246	21.8	54.5
91161	15.8	39.5
103833	15.04	37.6
104366	31.8	79.5
107129	21.2	53.0
107740	11.7	29.25
109020	16.3	40.75

Compounds were tested using the maximum high-test concentration possible based on the supplied stocks. Compounds were prepared at a 2X high-test concentration by combining drug stock with tissue culture media at a ratio of 5 µL of drug to 995 µL of media. This 2X high-test sample was subsequently serially diluted in tissue culture media using ½-log dilutions. This series of 2X concentrated drug was subsequently diluted 1:1 by combining with an equal volume of cells in media in the 96-well plates used for the assay.

Results for Testing Rekosh/Hammarskjöld Candidate Rev Inhibitors Against HIV-1 in Fresh Human PBMCs

Compound	HIV-1 BR/93/021			HIV-1 NL4-3			*HIV-1 ROJO**		
	IC_{50} (µM)	TC_{50} (µM)	TI (TC_{50}/IC_{50})	IC_{50} (µM)	TC_{50} (µM)	TI (TC_{50}/IC_{50})	*IC_{50} (µM)*	*TC_{50} (µM)*	*TI (TC_{50}/IC_{50})*
49611	2.41	>63.5	>26.35	5.14	>63.5	>12.35	*0.51*	*>63.5*	*>124.51*
73497	34.0	>90.6	>2.66	7.82	>90.6	>11.59	*12.2*	*>90.6*	*>7.43*
74377	>41.8	>41.8	N/A	>41.8	>41.8	N/A	*>41.8*	*>41.8*	*N/A*
74378	>43.2	>43.2	N/A	>43.2	>43.2	N/A	*>43.2*	*>43.2*	*N/A*
75168	2.14	>26.8	>12.52	16.9	>26.8	>1.59	*4.37*	*>26.8*	*>6.13*
89246	0.47	32.7	69.57	1.47	21.9	14.90	*1.19*	*21.9*	*18.40*
91161	1.94	23.3	12.01	2.28	13.5	5.92	*4.56*	*13.5*	*2.96*
103833	0.29	>37.6	>129.66	2.22	>37.6	>16.94	*18.6*	*>37.6*	*>2.02*
104366	0.64	24.9	38.91	0.92	22.0	23.91	*2.90*	*22.0*	*7.59*
107129	1.80	6.26	3.48	2.12	4.13	1.95	*2.17*	*4.13*	*1.90*
107740	0.20	18.8	94.00	1.61	14.4	8.94	*3.39*	*14.4*	*4.25*
109020	13.2	>40.8	>3.09	26.4	>40.8	>1.55	*15.6*	*>40.8*	*>2.62*
AZT	0.00247	>1.0	>404.86	0.00062	>1.0	>1612.90	*0.00316*	*>1.0*	*>316.46*
LeptB (historical Data)	–	–	–	0.00187	0.00190	1.02	–	–	–

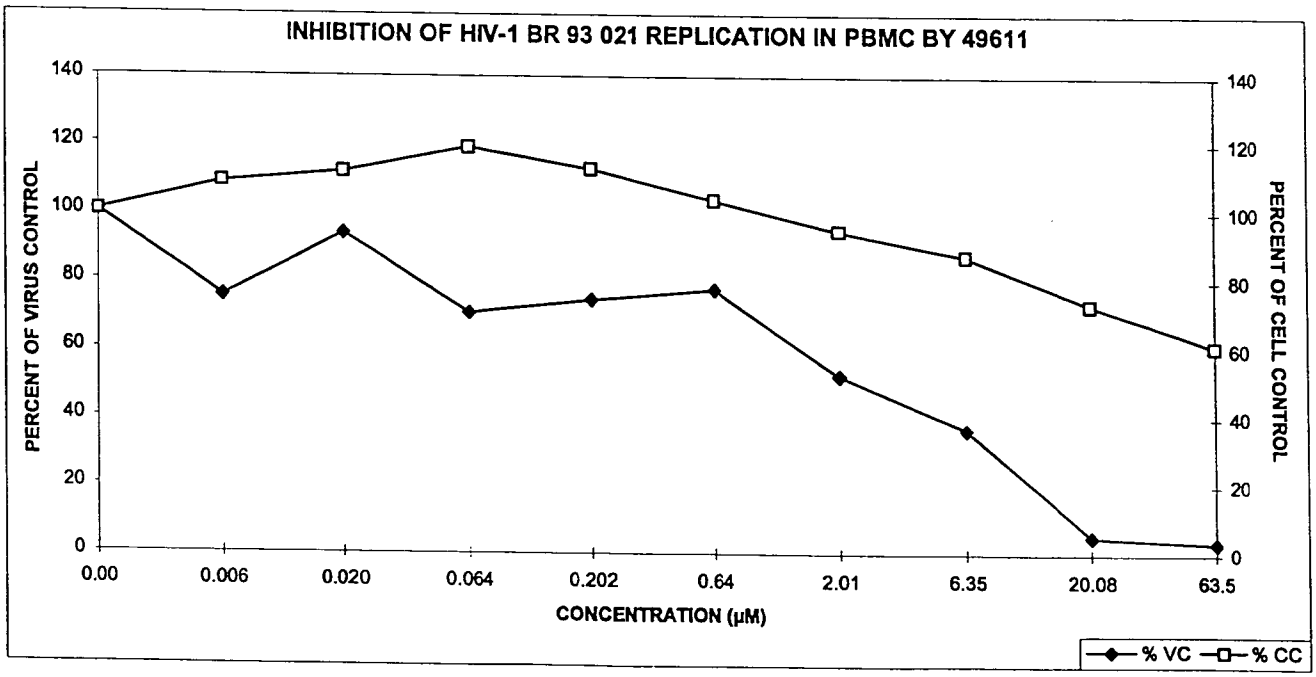
*HIV-1 ROJO Data included for comparison, however this assay contained significant variability in virus replication and should only be used for qualitative comparisons to other data.

INHIBITION OF HIV-1 BR 93 021 REPLICATION IN PBMC BY 49611

RT Values(cpm)										
CONC (µM)	0.00	0.006	0.020	0.064	0.202	0.64	2.01	6.35	20.08	63.5
SAMPLE 1	14637	11513	15424	11670	12674	11795	10630	6911	929	720
SAMPLE 2	15314	12111	16378	12483	11311	11062	5848	5390	728	569
SAMPLE 3	17039	11852	12266	9077	11054	13677	8176	4944	807	288
MEAN	15663.2	11825.3	14689.3	11076.7	11679.7	12178.0	8218.0	5748.3	821.3	525.7
% VC	100.0	75.5	93.8	70.7	74.6	77.7	52.5	36.7	5.2	3.4

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (µM)	0.00	0.006	0.020	0.064	0.202	0.64	2.01	6.35	20.08	63.5
SAMPLE 1	0.868	0.939	1.129	0.998	0.990	0.897	0.926	0.867	0.701	0.540
SAMPLE 2	0.958	0.963	0.939	1.266	1.165	0.968	0.983	0.760	0.718	0.586
SAMPLE 3	0.931	1.099	1.014	1.019	0.952	0.992	0.703	0.783	0.598	0.561
MEAN	0.919	1.000	1.027	1.094	1.036	0.952	0.871	0.803	0.672	0.562
% CC	100.0	108.8	111.8	119.1	112.7	103.6	94.7	87.4	73.1	61.1

IC50 (µM) =2.41	TC50 (µM) =>63.5	TI = >26.35



Client: IR&D
Investigator: Ptak
Setup Date: 04/02/04

Virus/Strain: HIV-1 / BR 93 021
Virus Date/Titer: 10/18/99, 3.3 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY 49611

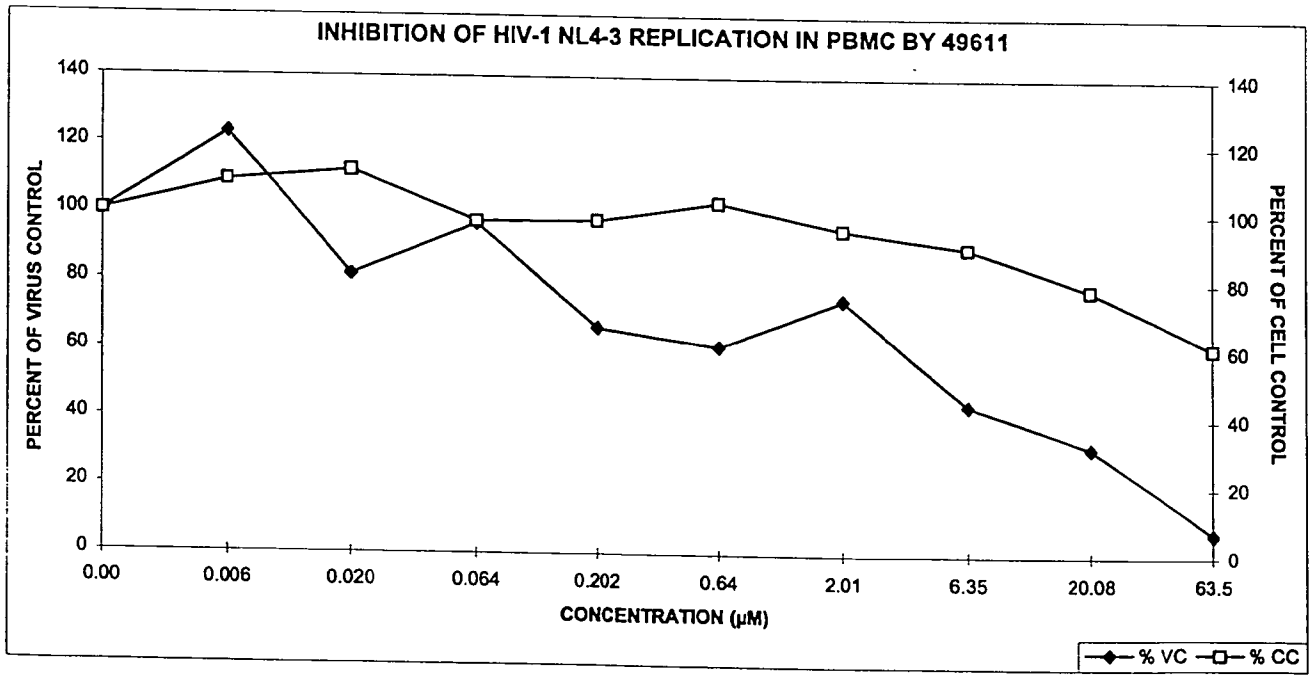
RT Values(cpm)										
CONC (μM)	0.00	0.006	0.020	0.064	0.202	0.64	2.01	6.35	20.08	63.5
SAMPLE 1	39777	39910	33022	36199	25437	18082	21866	19031	15891	728
SAMPLE 2	33324	48011	19495	38056	22079	12374	20833	16426	10495	1852
SAMPLE 3	34624	44745	35557	30008	24323	35589	38262	12298	8029	4700
MEAN	35908.2	44222.0	29358.0	34754.3	23946.3	22015.0	26987.0	15918.3	11471.7	2426.7
% VC	100.0	123.2	81.8	96.8	66.7	61.3	75.2	44.3	31.9	6.8

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.006	0.020	0.064	0.202	0.64	2.01	6.35	20.08	63.5
SAMPLE 1	0.761	0.638	0.779	0.648	0.742	0.712	0.677	0.713	0.508	0.367
SAMPLE 2	0.637	0.728	0.723	0.616	0.562	0.670	0.576	0.541	0.516	0.367
SAMPLE 3	0.604	0.820	0.742	0.687	0.655	0.686	0.659	0.556	0.542	0.490
MEAN	0.667	0.728	0.748	0.650	0.653	0.689	0.637	0.604	0.522	0.408
% CC	100.0	109.2	112.1	97.4	97.8	103.3	95.5	90.4	78.2	61.1

IC50 (μM) =5.14

TC50 (μM) =>63.5

TI = >12.35



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

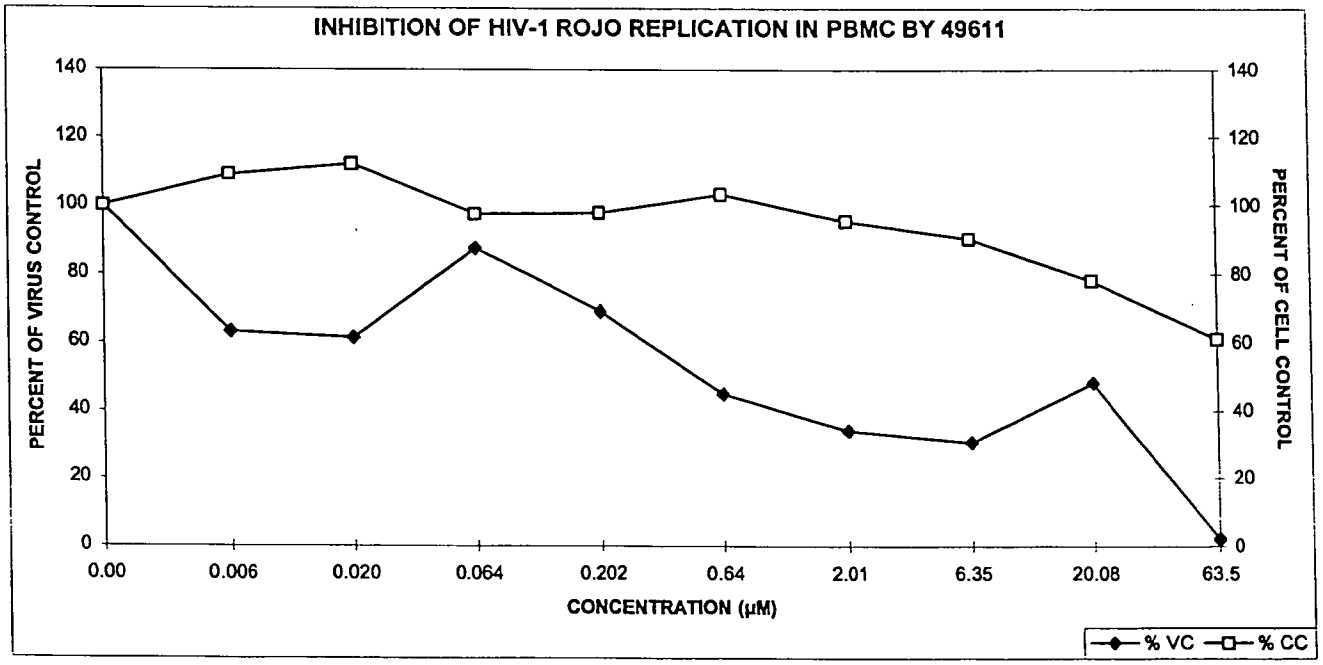
Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 4/25/03, 1.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 ROJO REPLICATION IN PBMC BY 49611

RT Values(cpm)										
CONC (µM)	0.00	0.006	0.020	0.064	0.202	0.64	2.01	6.35	20.08	63.5
SAMPLE 1	79707	89103	64976	61827	48932	41605	1844	15530	36409	1513
SAMPLE 2	84041	41714	33313	84689	42954	55999	17071	25731	41953	2652
SAMPLE 3	64377	13620	41864	53230	66033	5211	59426	28916	32104	612
MEAN	76041.5	48145.7	46717.7	66582.0	52639.7	34271.7	26113.7	23392.3	36822.0	1592.3
% VC	100.0	63.3	61.4	87.6	69.2	45.1	34.3	30.8	48.4	2.1

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (µM)	0.00	0.006	0.020	0.064	0.202	0.64	2.01	6.35	20.08	63.5
SAMPLE 1	0.761	0.638	0.779	0.648	0.742	0.712	0.677	0.713	0.508	0.367
SAMPLE 2	0.637	0.728	0.723	0.616	0.562	0.670	0.576	0.541	0.516	0.367
SAMPLE 3	0.604	0.820	0.742	0.687	0.655	0.686	0.659	0.556	0.542	0.490
MEAN	0.667	0.728	0.748	0.650	0.653	0.689	0.637	0.604	0.522	0.408
% CC	100.0	109.2	112.1	97.4	97.8	103.3	95.5	90.4	78.2	61.1

IC50 (µM) =0.51	TC50 (µM) =>63.5	TI = >124.51



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

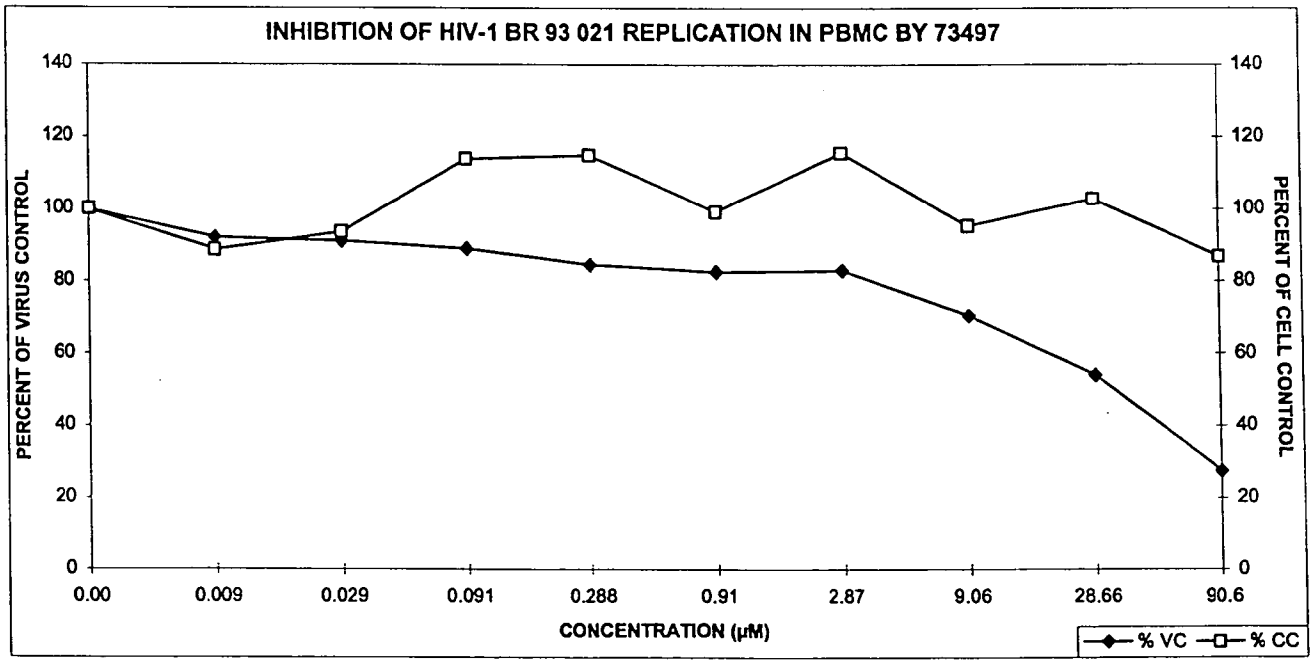
Virus/Strain: HIV-1 / ROJO
Virus Date/Titer: 12/16/02, 3.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 BR 93 021 REPLICATION IN PBMC BY 73497

RT Values(cpm)										
CONC (µM)	0.00	0.009	0.029	0.091	0.288	0.91	2.87	9.06	28.66	90.6
SAMPLE 1	14637	12562	16475	15991	14327	12072	13877	9383	8713	2718
SAMPLE 2	15314	14361	13590	12249	14880	9130	12958	9687	9357	6110
SAMPLE 3	17039	16317	12753	13585	10511	17653	12212	14101	7283	4066
MEAN	15663.2	14413.3	14272.7	13941.7	13239.3	12951.7	13015.7	11057.0	8451.0	4298.0
% VC	100.0	92.0	91.1	89.0	84.5	82.7	83.1	70.6	54.0	27.4

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (µM)	0.00	0.009	0.029	0.091	0.288	0.91	2.87	9.06	28.66	90.6
SAMPLE 1	0.868	0.828	0.829	1.181	1.132	0.793	0.799	0.756	0.809	0.688
SAMPLE 2	0.958	0.852	0.818	0.840	0.918	1.014	1.252	1.147	0.931	0.692
SAMPLE 3	0.931	0.766	0.933	1.116	1.116	0.929	1.135	0.725	1.092	1.010
MEAN	0.919	0.815	0.860	1.045	1.055	0.912	1.062	0.876	0.944	0.797
% CC	100.0	88.7	93.6	113.8	114.8	99.2	115.5	95.3	102.7	86.7

IC50 (µM) =34.0	TC50 (µM) =>90.6	TI = >2.66



Client: IR&D
Investigator: Ptak
Setup Date: 04/02/04

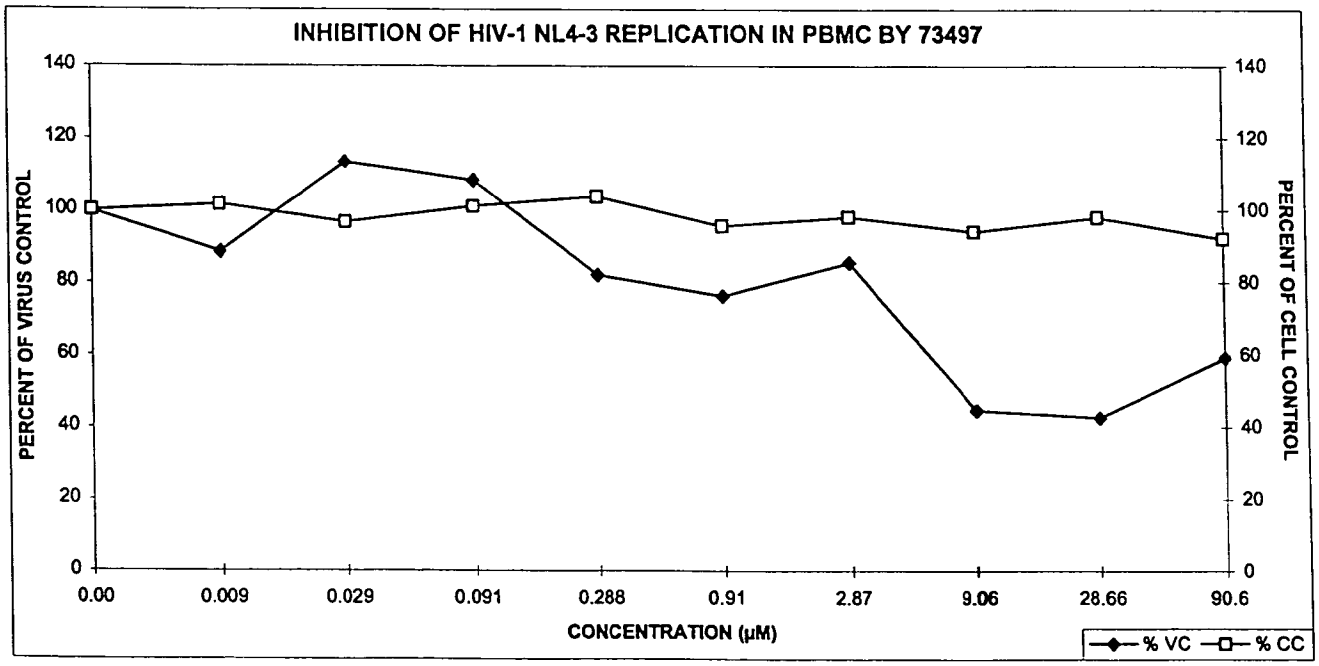
Virus/Strain: HIV-1 / BR 93 021
Virus Date/Titer: 10/18/99, 3.3 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY 73497

RT Values(cpm)										
CONC (µM)	0.00	0.009	0.029	0.091	0.288	0.91	2.87	9.06	28.66	90.6
SAMPLE 1	39777	33329	38235	37370	35582	34293	29310	12855	18921	25858
SAMPLE 2	33324	26541	44611	45089	25475	24536	31073	8449	11505	16428
SAMPLE 3	34624	35459	39269	34175	27451	23463	31802	26951	15554	21621
MEAN	35908.2	31776.3	40705.0	38878.0	29502.7	27430.7	30728.3	16085.0	15326.7	21302.3
% VC	100.0	88.5	113.4	108.3	82.2	76.4	85.6	44.8	42.7	59.3

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (µM)	0.00	0.009	0.029	0.091	0.288	0.91	2.87	9.06	28.66	90.6
SAMPLE 1	0.761	0.697	0.641	0.639	0.585	0.567	0.639	0.541	0.573	0.628
SAMPLE 2	0.637	0.756	0.708	0.736	0.819	0.772	0.685	0.721	0.741	0.661
SAMPLE 3	0.604	0.582	0.587	0.647	0.674	0.577	0.642	0.622	0.648	0.555
MEAN	0.667	0.678	0.645	0.674	0.693	0.638	0.655	0.628	0.654	0.614
% CC	100.0	101.6	96.7	101.0	103.8	95.7	98.2	94.1	98.0	92.1

IC50 (µM) =7.82	TC50 (µM) =>90.6	TI = >11.59



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

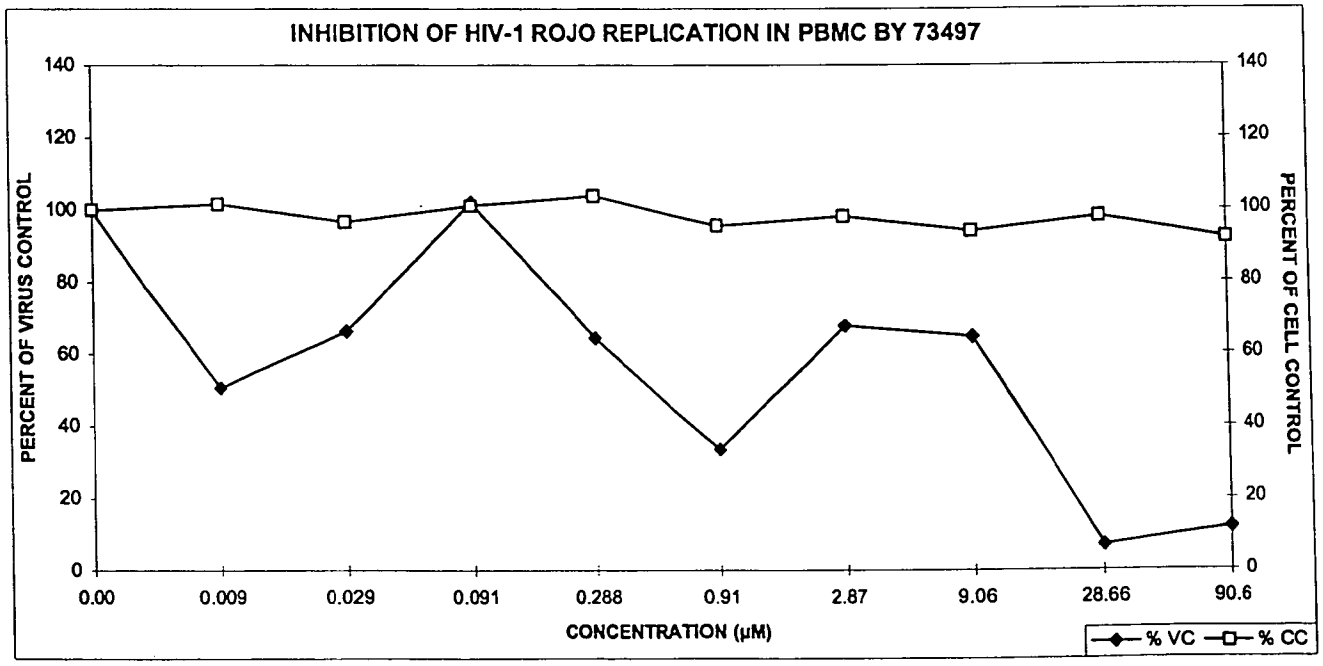
Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 4/25/03, 1.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 ROJO REPLICATION IN PBMC BY 73497

RT Values(cpm)										
CONC (μM)	0.00	0.009	0.029	0.091	0.288	0.91	2.87	9.06	28.66	90.6
SAMPLE 1	79707	20074	50030	86124	62707	23766	15672	72576	9137	945
SAMPLE 2	84041	54668	34395	64114	42361	12909	54680	850	396	9592
SAMPLE 3	64377	41248	67020	82451	42282	40068	84341	74573	6572	16713
MEAN	76041.5	38663.3	50481.7	77563.0	49116.7	25581.0	51564.3	49333.0	5368.3	9083.3
% VC	100.0	50.8	66.4	102.0	64.6	33.6	67.8	64.9	7.1	11.9

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.009	0.029	0.091	0.288	0.91	2.87	9.06	28.66	90.6
SAMPLE 1	0.761	0.697	0.641	0.639	0.585	0.567	0.639	0.541	0.573	0.628
SAMPLE 2	0.637	0.756	0.708	0.736	0.819	0.772	0.685	0.721	0.741	0.661
SAMPLE 3	0.604	0.582	0.587	0.647	0.674	0.577	0.642	0.622	0.648	0.555
MEAN	0.667	0.678	0.645	0.674	0.693	0.638	0.655	0.628	0.654	0.614
% CC	100.0	101.6	96.7	101.0	103.8	95.7	98.2	94.1	98.0	92.1

IC50 (μM) =12.2	TC50 (μM) =>90.6	TI = >7.43



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

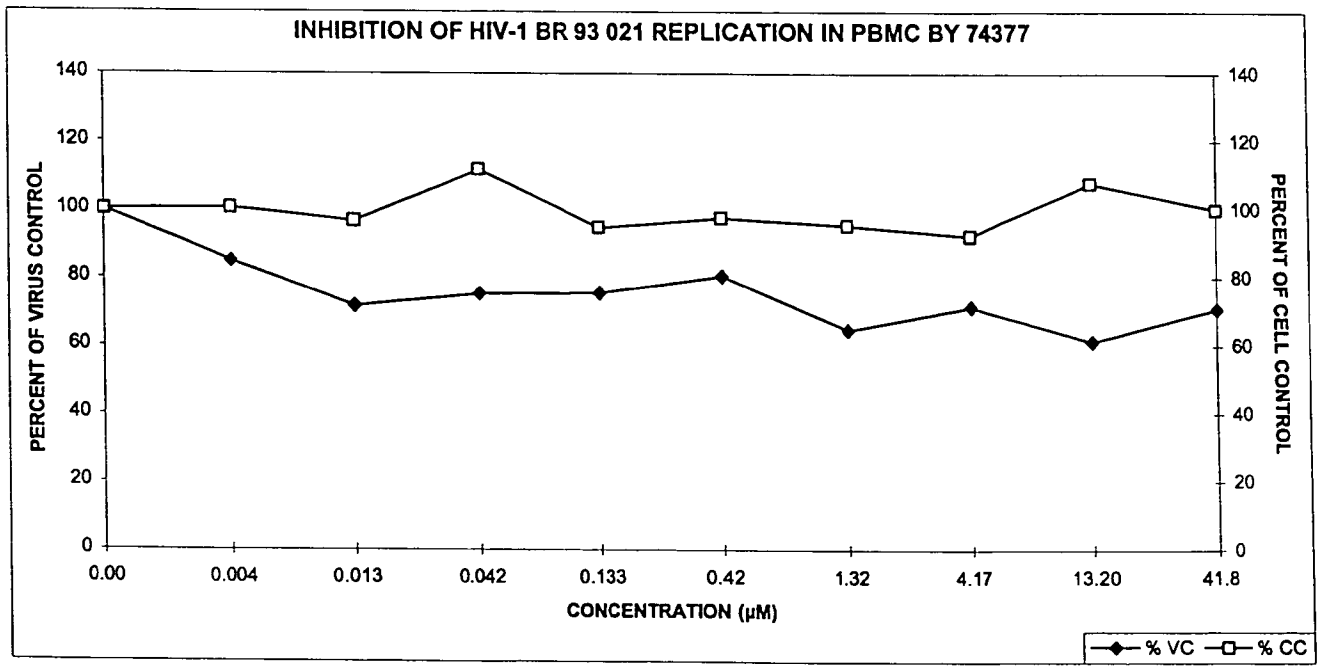
Virus/Strain: HIV-1 / ROJO
Virus Date/Titer: 12/16/02, 3.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 BR 93 021 REPLICATION IN PBMC BY 74377

RT Values(cpm)										
CONC (μM)	0.00	0.004	0.013	0.042	0.133	0.42	1.32	4.17	13.20	41.8
SAMPLE 1	13361	14293	9797	12483	14235	12162	8609	11238	8887	13655
SAMPLE 2	14217	11246	11107	10073	7503	9810	8470	9673	9132	7510
SAMPLE 3	13903	9709	8880	8697	9612	11451	9751	8772	7388	8231
MEAN	13826.7	11749.3	9928.0	10417.7	10450.0	11141.0	8943.3	9894.3	8469.0	9798.7
% VC	100.0	85.0	71.8	75.3	75.6	80.6	64.7	71.6	61.3	70.9

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.004	0.013	0.042	0.133	0.42	1.32	4.17	13.20	41.8
SAMPLE 1	0.793	0.810	0.793	1.267	0.850	0.830	0.783	0.736	0.993	0.807
SAMPLE 2	0.809	0.836	0.788	0.750	0.725	0.816	0.765	0.779	0.803	0.842
SAMPLE 3	0.832	0.800	0.773	0.704	0.732	0.728	0.774	0.734	0.825	0.784
MEAN	0.812	0.815	0.785	0.907	0.769	0.791	0.774	0.750	0.874	0.811
% CC	100.0	100.4	96.7	111.7	94.8	97.5	95.4	92.4	107.7	100.0

IC50 (μM) =>41.8	TC50 (μM) =>41.8	TI = N/A



Client: IR&D
Investigator: Ptak
Setup Date: 04/02/04

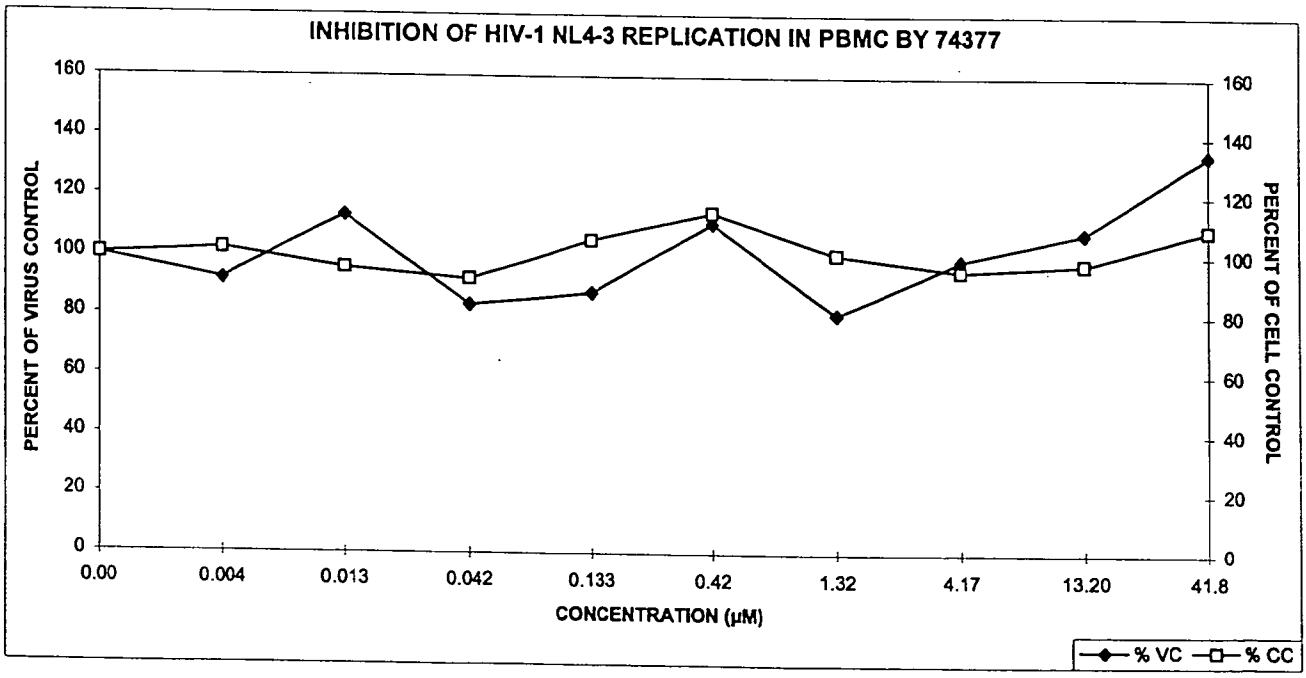
Virus/Strain: HIV-1 / BR 93 021
Virus Date/Titer: 10/18/99, 3.3 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY 74377

RT Values(cpm)										
CONC (μM)	0.00	0.004	0.013	0.042	0.133	0.42	1.32	4.17	13.20	41.8
SAMPLE 1	28873	18671	40971	32776	38929	39685	8257	28007	30885	58054
SAMPLE 2	27831	29377	40665	28232	16118	21053	41767	38591	33205	43585
SAMPLE 3	42164	42746	30145	21403	31535	48858	29501	30941	42160	30864
MEAN	32955.8	30264.7	37260.3	27470.3	28860.7	36532.0	26508.3	32513.0	35416.7	44167.7
% VC	100.0	91.8	113.1	83.4	87.6	110.9	80.4	98.7	107.5	134.0

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.004	0.013	0.042	0.133	0.42	1.32	4.17	13.20	41.8
SAMPLE 1	0.557	0.626	0.563	0.614	0.667	0.742	0.678	0.622	0.663	0.746
SAMPLE 2	0.704	0.663	0.631	0.539	0.609	0.796	0.594	0.563	0.608	0.640
SAMPLE 3	0.673	0.683	0.656	0.630	0.753	0.674	0.671	0.654	0.609	0.719
MEAN	0.644	0.657	0.616	0.594	0.676	0.737	0.648	0.613	0.627	0.702
% CC	100.0	102.0	95.6	92.2	105.0	114.4	100.5	95.1	97.2	108.9

IC50 (μM) =>41.8	TC50 (μM) =>41.8	TI = N/A



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

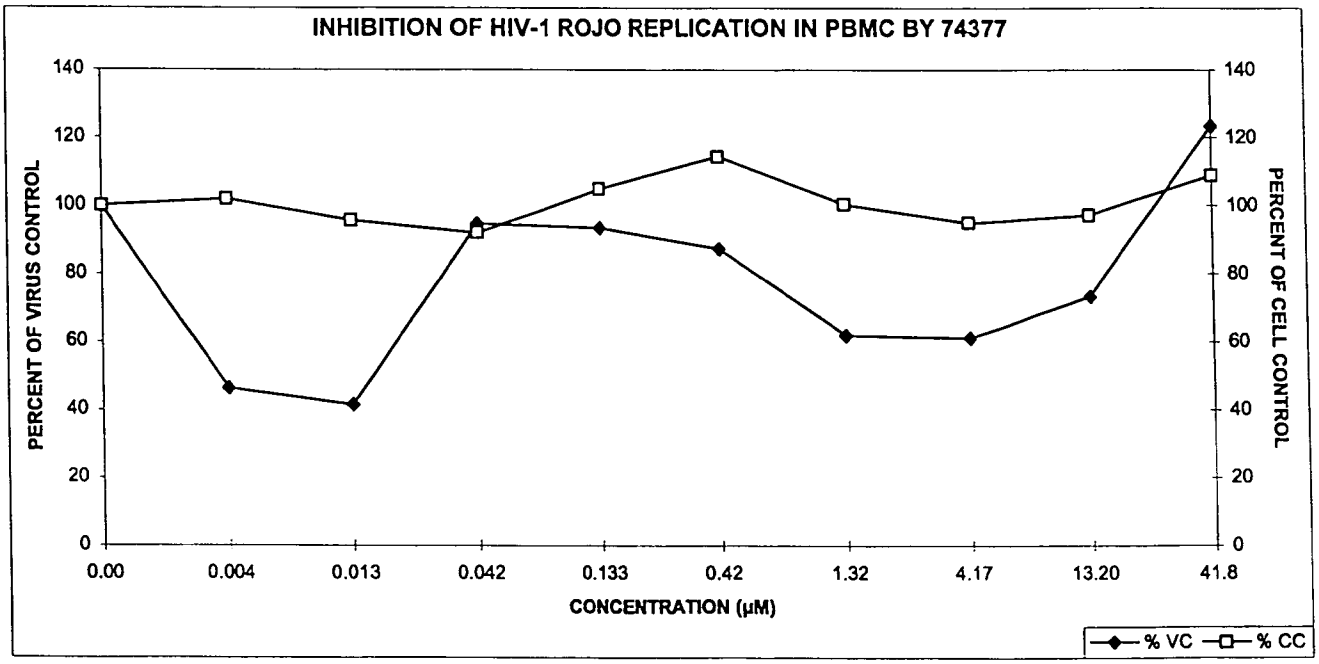
Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 4/25/03, 1.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 ROJO REPLICATION IN PBMC BY 74377

RT Values(cpm)										
CONC (μM)	0.00	0.004	0.013	0.042	0.133	0.42	1.32	4.17	13.20	41.8
SAMPLE 1	80292	65211	3450	92317	77115	58099	49741	35780	61456	37230
SAMPLE 2	33041	12977	27436	63431	63221	53291	25482	51182	28646	91356
SAMPLE 3	58287	1654	40710	6976	20275	38796	31277	18171	35633	82994
MEAN	57206.5	26614.0	23865.3	54241.3	53537.0	50062.0	35500.0	35044.3	41911.7	70526.7
% VC	100.0	46.5	41.7	94.8	93.6	87.5	62.1	61.3	73.3	123.3

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.004	0.013	0.042	0.133	0.42	1.32	4.17	13.20	41.8
SAMPLE 1	0.557	0.626	0.563	0.614	0.667	0.742	0.678	0.622	0.663	0.746
SAMPLE 2	0.704	0.663	0.631	0.539	0.609	0.796	0.594	0.563	0.608	0.640
SAMPLE 3	0.673	0.683	0.656	0.630	0.753	0.674	0.671	0.654	0.609	0.719
MEAN	0.644	0.657	0.616	0.594	0.676	0.737	0.648	0.613	0.627	0.702
% CC	100.0	102.0	95.6	92.2	105.0	114.4	100.5	95.1	97.2	108.9

IC50 (μM) =>41.8	TC50 (μM) =>41.8	TI = N/A



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

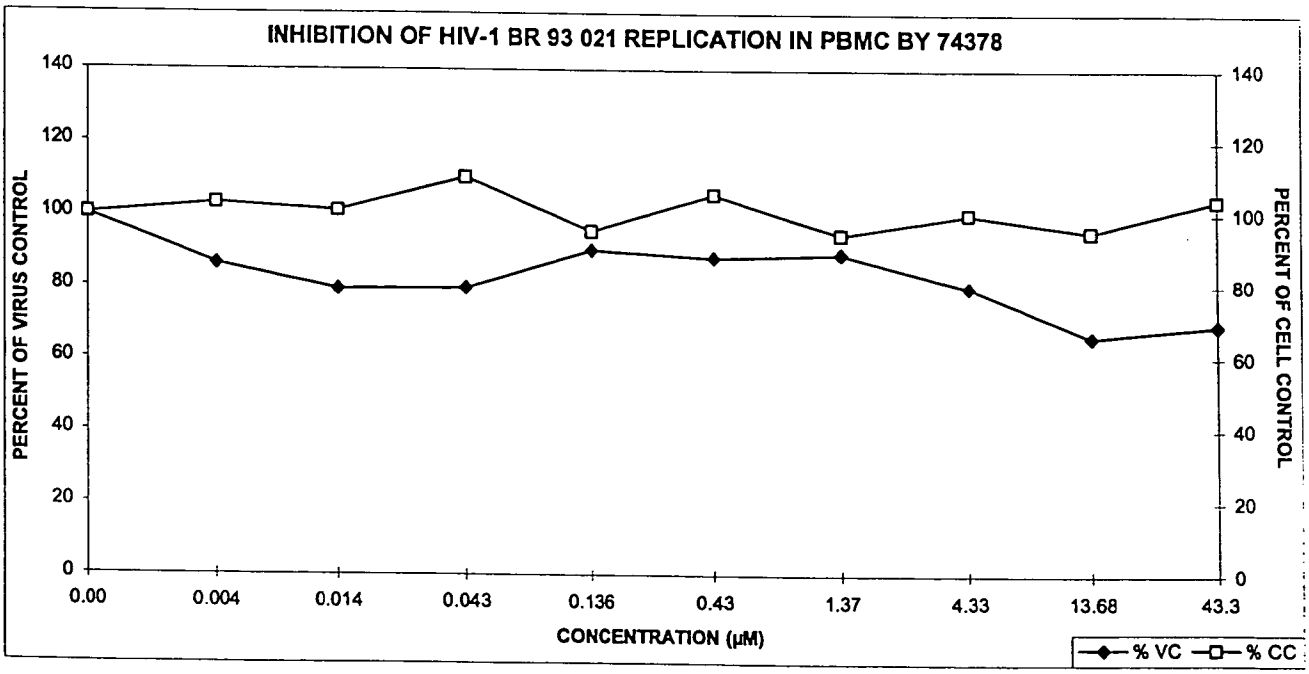
Virus/Strain: HIV-1 / ROJO
Virus Date/Titer: 12/16/02, 3.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 BR 93 021 REPLICATION IN PBMC BY 74378

RT Values(cpm)										
CONC (μM)	0.00	0.004	0.014	0.043	0.136	0.43	1.37	4.33	13.68	43.3
SAMPLE 1	13361	9443	11354	9441	10031	11775	9787	10171	8140	9357
SAMPLE 2	14217	12687	11043	11620	13165	9381	13099	9812	9555	7173
SAMPLE 3	13903	13664	10473	11967	14215	15428	14149	13243	9709	12136
MEAN	13826.7	11931.3	10956.7	11009.3	12470.3	12194.7	12345.0	11075.3	9134.7	9555.3
% VC	100.0	86.3	79.2	79.6	90.2	88.2	89.3	80.1	66.1	69.1

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.004	0.014	0.043	0.136	0.43	1.37	4.33	13.68	43.3
SAMPLE 1	0.793	0.873	0.803	0.816	0.766	0.792	0.710	0.783	0.742	0.770
SAMPLE 2	0.809	0.812	0.820	1.038	0.762	0.899	0.802	0.835	0.770	0.887
SAMPLE 3	0.832	0.821	0.832	0.825	0.791	0.878	0.787	0.820	0.803	0.868
MEAN	0.812	0.835	0.818	0.893	0.773	0.856	0.766	0.813	0.772	0.841
% CC	100.0	102.9	100.8	110.1	95.2	105.5	94.4	100.2	95.1	103.7

IC50 (μM) =>43.2	TC50 (μM) =>43.2	TI = N/A



Client: IR&D
Investigator: Ptak
Setup Date: 04/02/04

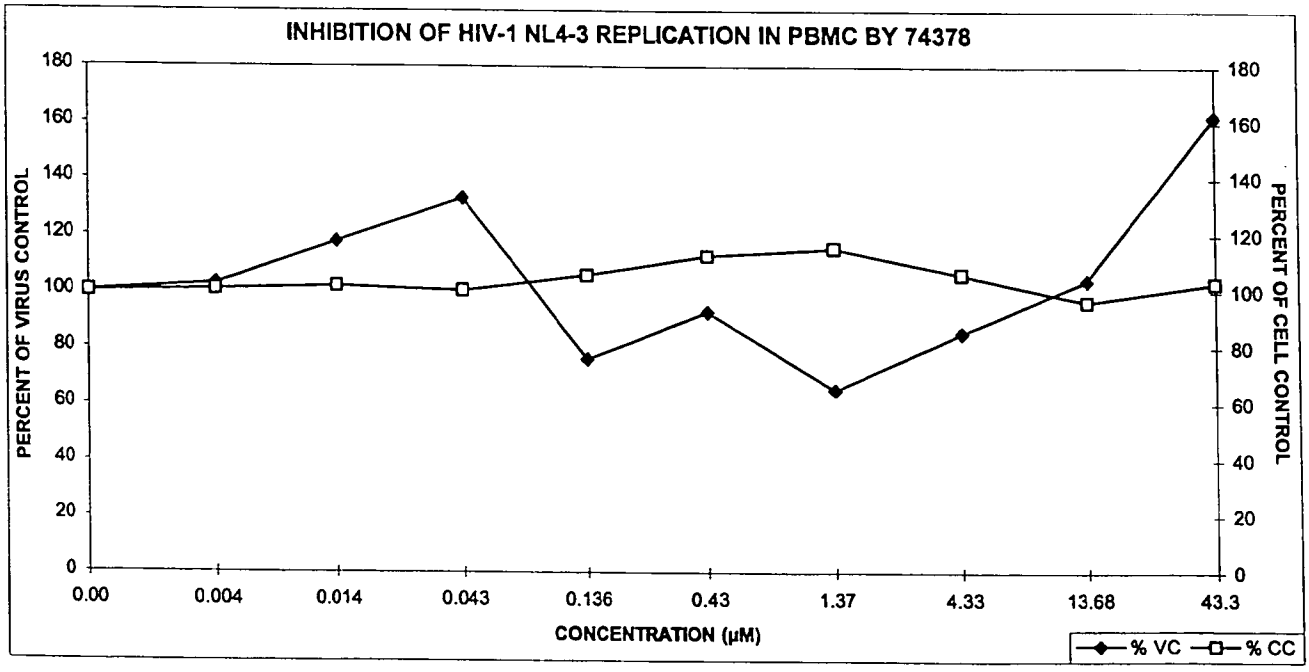
Virus/Strain: HIV-1 / BR 93 021
Virus Date/Titer: 10/18/99, 3.3 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY 74378

RT Values(cpm)										
CONC (µM)	0.00	0.004	0.014	0.043	0.136	0.43	1.37	4.33	13.68	43.3
SAMPLE 1	28873	37415	25411	40517	19394	35123	17749	39958	31444	59654
SAMPLE 2	27831	25479	39704	46748	28933	29657	21263	23171	39198	49298
SAMPLE 3	42164	38785	51147	44181	26970	27177	25579	21496	32397	51337
MEAN	32955.8	33893.0	38754.0	43815.3	25099.0	30652.3	21530.3	28208.3	34346.3	53429.7
% VC	100.0	102.8	117.6	133.0	76.2	93.0	65.3	85.6	104.2	162.1

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (µM)	0.00	0.004	0.014	0.043	0.136	0.43	1.37	4.33	13.68	43.3
SAMPLE 1	0.557	0.641	0.627	0.648	0.615	0.613	0.792	0.660	0.589	0.636
SAMPLE 2	0.704	0.679	0.696	0.697	0.737	0.934	0.791	0.738	0.668	0.620
SAMPLE 3	0.673	0.630	0.647	0.597	0.693	0.633	0.651	0.659	0.611	0.736
MEAN	0.644	0.650	0.657	0.647	0.682	0.727	0.744	0.685	0.623	0.664
% CC	100.0	100.9	101.9	100.4	105.8	112.8	115.5	106.4	96.6	103.1

IC50 (µM) =>43.2	TC50 (µM) =>43.2	TI = N/A



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 4/25/03, 1.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 ROJO REPLICATION IN PBMC BY 74378

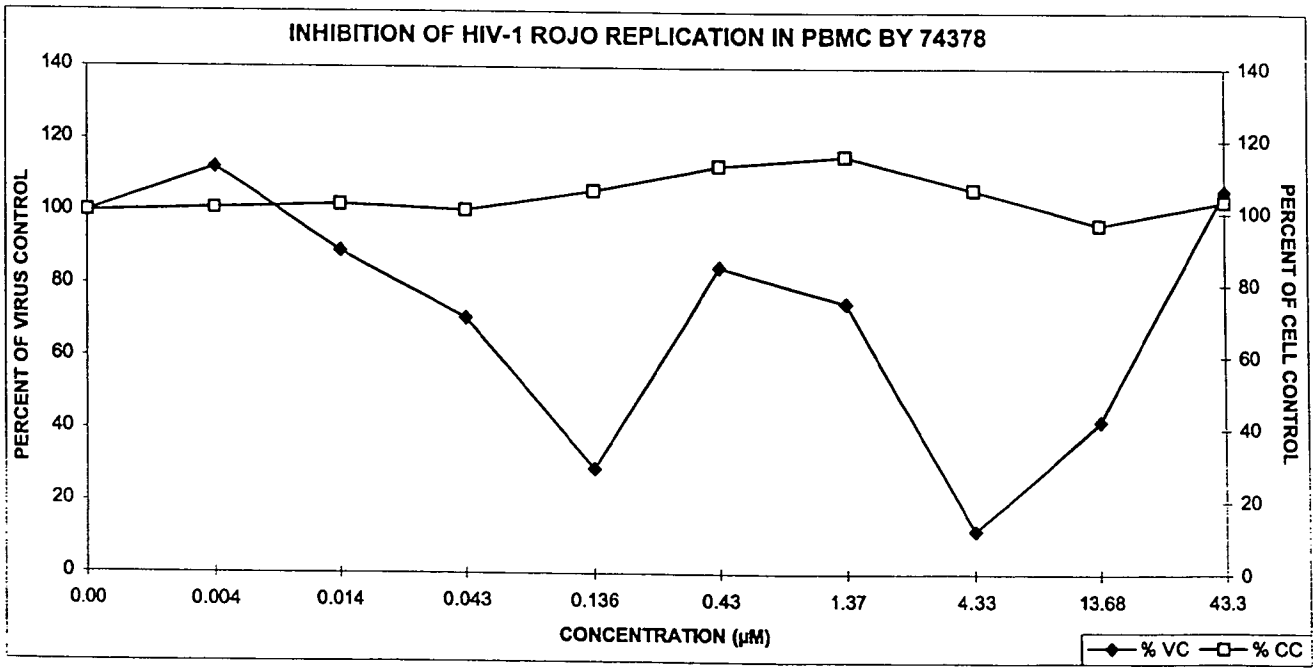
RT Values(cpm)										
CONC (µM)	0.00	0.004	0.014	0.043	0.136	0.43	1.37	4.33	13.68	43.3
SAMPLE 1	80292	88179	47675	51458	16226	51880	40859	11011	20109	48046
SAMPLE 2	33041	20966	28454	43672	25468	50922	76532	7393	36109	65079
SAMPLE 3	58287	83430	77064	26369	8213	43027	11443	2344	16514	68682
MEAN	57206.5	64191.7	51064.3	40499.7	16635.7	48609.7	42944.7	6916.0	24244.0	60602.3
% VC	100.0	112.2	89.3	70.8	29.1	85.0	75.1	12.1	42.4	105.9

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (µM)	0.00	0.004	0.014	0.043	0.136	0.43	1.37	4.33	13.68	43.3
SAMPLE 1	0.557	0.641	0.627	0.648	0.615	0.613	0.792	0.660	0.589	0.636
SAMPLE 2	0.704	0.679	0.696	0.697	0.737	0.934	0.791	0.738	0.668	0.620
SAMPLE 3	0.673	0.630	0.647	0.597	0.693	0.633	0.651	0.659	0.611	0.736
MEAN	0.644	0.650	0.657	0.647	0.682	0.727	0.744	0.685	0.623	0.664
% CC	100.0	100.9	101.9	100.4	105.8	112.8	115.5	106.4	96.6	103.1

IC50 (µM) =>43.2

TC50 (µM) =>43.2

TI = N/A



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

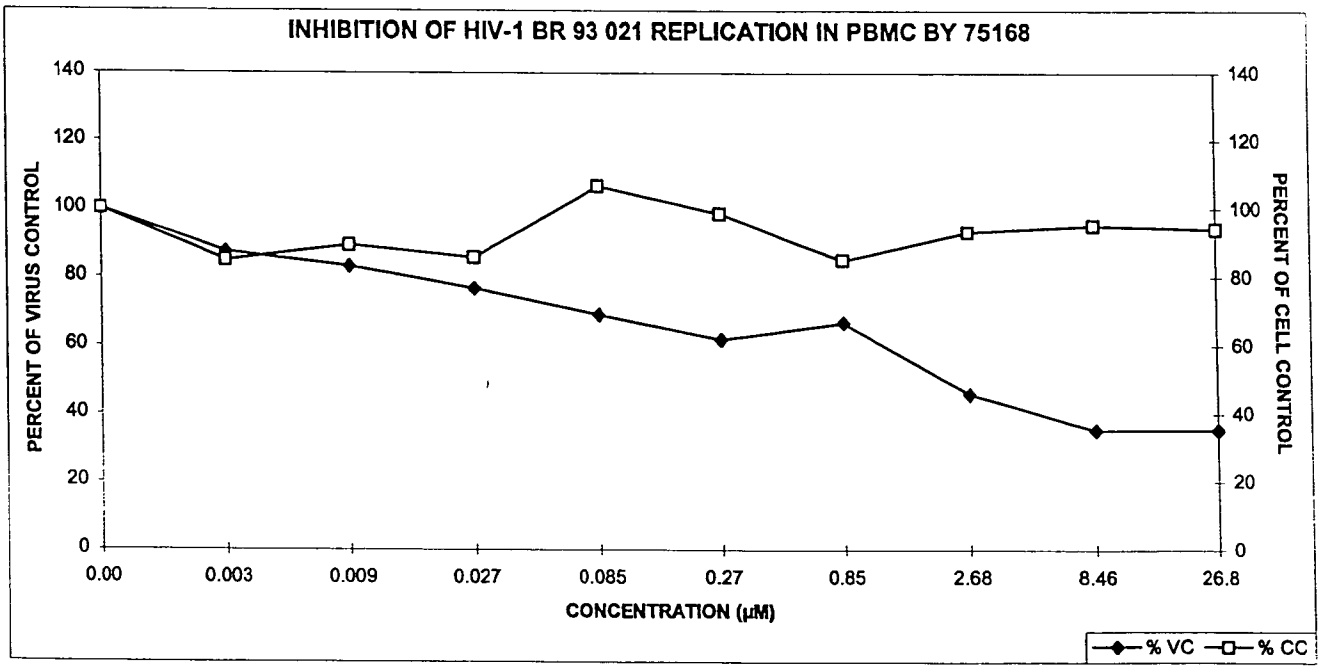
Virus/Strain: HIV-1 / ROJO
Virus Date/Titer: 12/16/02, 3.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 BR 93 021 REPLICATION IN PBMC BY 75168

RT Values(cpm)										
CONC (μM)	0.00	0.003	0.009	0.027	0.085	0.27	0.85	2.68	8.46	26.8
SAMPLE 1	16603	16651	16281	12681	11318	11325	10366	7243	6219	6140
SAMPLE 2	13824	12562	12607	10483	11494	9670	9363	5924	3293	3776
SAMPLE 3	13535	9257	7735	10593	7634	6250	9721	7012	6025	5607
MEAN	14653.8	12823.3	12207.7	11252.3	10148.7	9081.7	9816.7	6726.3	5179.0	5174.3
% VC	100.0	87.5	83.3	76.8	69.3	62.0	67.0	45.9	35.3	35.3

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.003	0.009	0.027	0.085	0.27	0.85	2.68	8.46	26.8
SAMPLE 1	0.838	0.780	0.751	0.773	0.877	0.835	0.756	0.875	0.880	0.815
SAMPLE 2	0.833	0.788	0.761	0.750	0.686	0.695	0.690	0.763	0.792	0.797
SAMPLE 3	0.922	0.637	0.807	0.703	1.206	1.028	0.762	0.782	0.799	0.826
MEAN	0.864	0.735	0.773	0.742	0.923	0.853	0.736	0.807	0.824	0.813
% CC	100.0	85.0	89.4	85.9	106.7	98.7	85.1	93.3	95.3	94.0

IC50 (μM) =2.14	TC50 (μM) =>26.8	TI = >12.52



Client: IR&D
Investigator: Ptak
Setup Date: 04/02/04

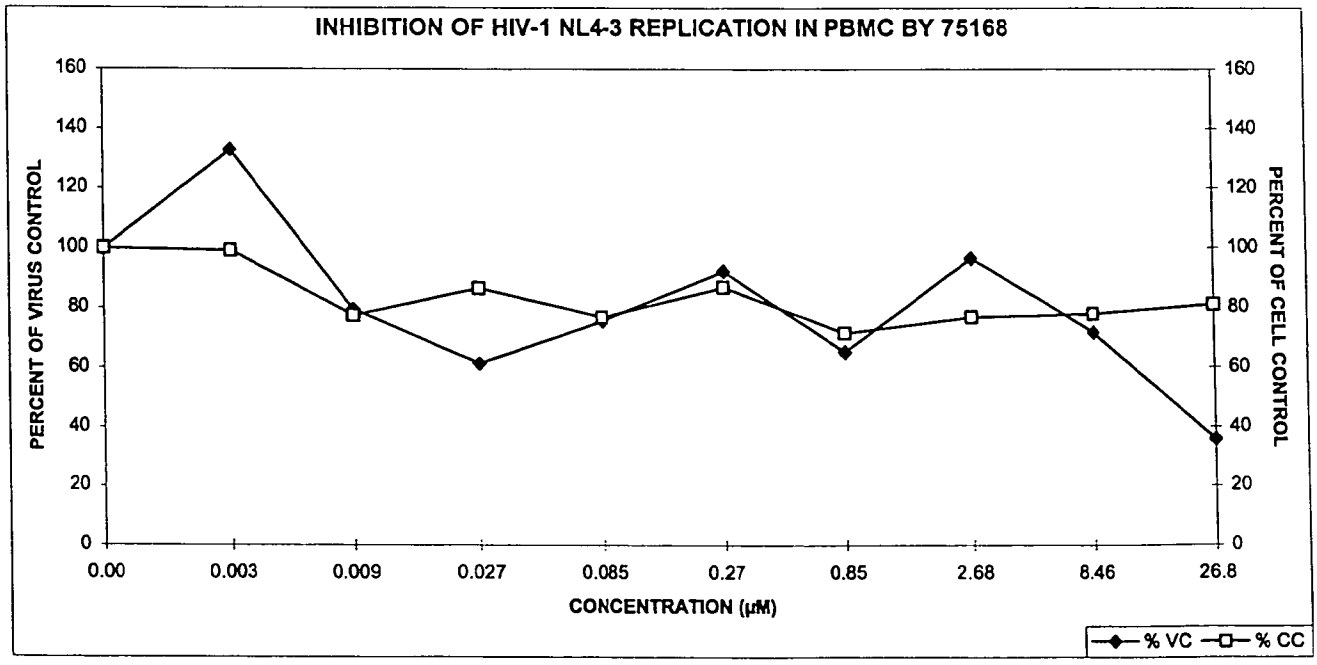
Virus/Strain: HIV-1 / BR 93 021
Virus Date/Titer: 10/18/99, 3.3 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY 75168

RT Values(cpm)										
CONC (µM)	0.00	0.003	0.009	0.027	0.085	0.27	0.85	2.68	8.46	26.8
SAMPLE 1	17547	38117	11771	14761	9751	28561	12857	31721	21276	4396
SAMPLE 2	39386	43541	28183	21029	31175	23618	24132	23832	28246	16216
SAMPLE 3	44311	52804	40570	26258	35588	41237	29179	42291	23089	15545
MEAN	33747.8	44820.7	26841.3	20682.7	25504.7	31138.7	22056.0	32614.7	24203.7	12052.3
% VC	100.0	132.8	79.5	61.3	75.6	92.3	65.4	96.6	71.7	35.7

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (µM)	0.00	0.003	0.009	0.027	0.085	0.27	0.85	2.68	8.46	26.8
SAMPLE 1	0.762	0.697	0.580	0.609	0.530	0.593	0.497	0.544	0.602	0.626
SAMPLE 2	0.806	0.764	0.632	0.741	0.609	0.785	0.548	0.563	0.573	0.609
SAMPLE 3	0.707	0.796	0.552	0.617	0.608	0.597	0.585	0.648	0.596	0.606
MEAN	0.758	0.752	0.588	0.656	0.582	0.658	0.543	0.585	0.591	0.614
% CC	100.0	99.2	77.5	86.5	76.8	86.8	71.7	77.1	77.9	80.9

IC50 (µM) =16.9	TC50 (µM) =>26.8	TI = >1.59



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

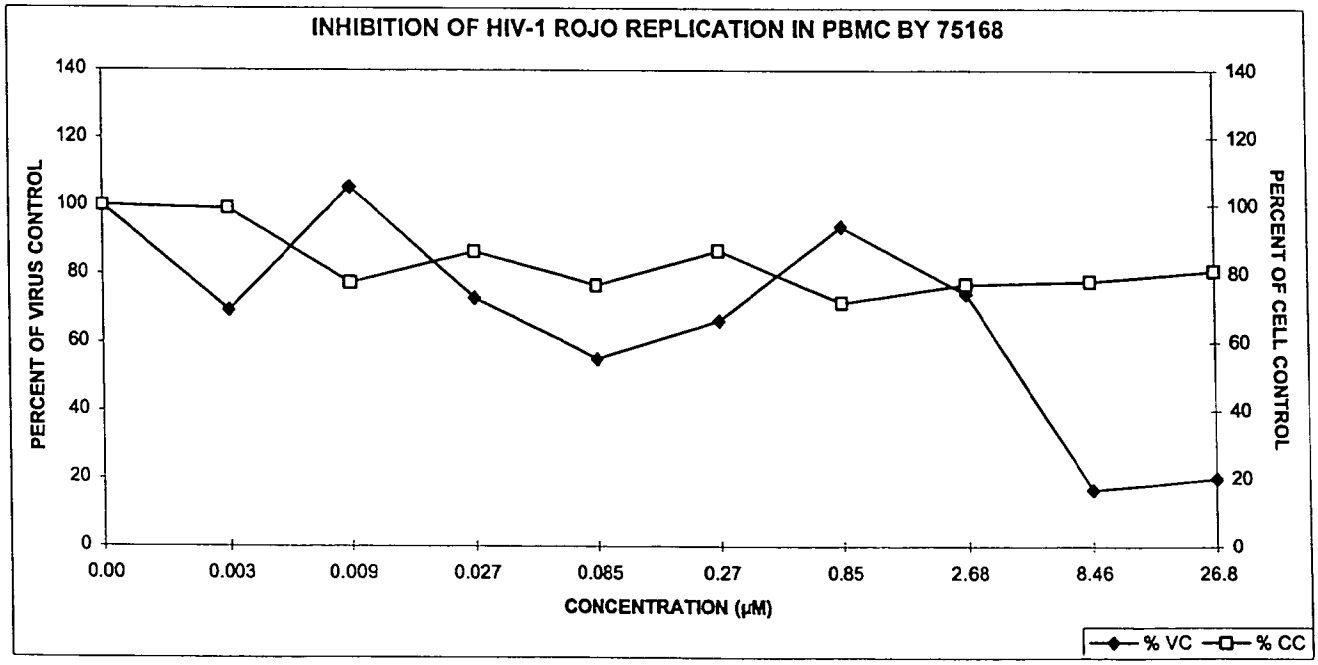
Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 4/25/03, 1.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 ROJO REPLICATION IN PBMC BY 75168

RT Values(cpm)										
CONC (μM)	0.00	0.003	0.009	0.027	0.085	0.27	0.85	2.68	8.46	26.8
SAMPLE 1	77999	19940	72194	43636	36089	33576	28844	42843	4194	26676
SAMPLE 2	36238	41261	42109	35824	22093	42750	68597	46291	12962	461
SAMPLE 3	46860	50804	55583	38130	30810	30748	53857	30824	9932	5088
MEAN	53698.8	37335.0	56628.7	39196.7	29664.0	35691.3	50432.7	39986.0	9029.3	10741.7
% VC	100.0	69.5	105.5	73.0	55.2	66.5	93.9	74.5	16.8	20.0

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.003	0.009	0.027	0.085	0.27	0.85	2.68	8.46	26.8
SAMPLE 1	0.762	0.697	0.580	0.609	0.530	0.593	0.497	0.544	0.602	0.626
SAMPLE 2	0.806	0.764	0.632	0.741	0.609	0.785	0.548	0.563	0.573	0.609
SAMPLE 3	0.707	0.796	0.552	0.617	0.608	0.597	0.585	0.648	0.596	0.606
MEAN	0.758	0.752	0.588	0.656	0.582	0.658	0.543	0.585	0.591	0.614
% CC	100.0	99.2	77.5	86.5	76.8	86.8	71.7	77.1	77.9	80.9

IC50 (μM) =4.37	TC50 (μM) =>26.8	TI = >6.13



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

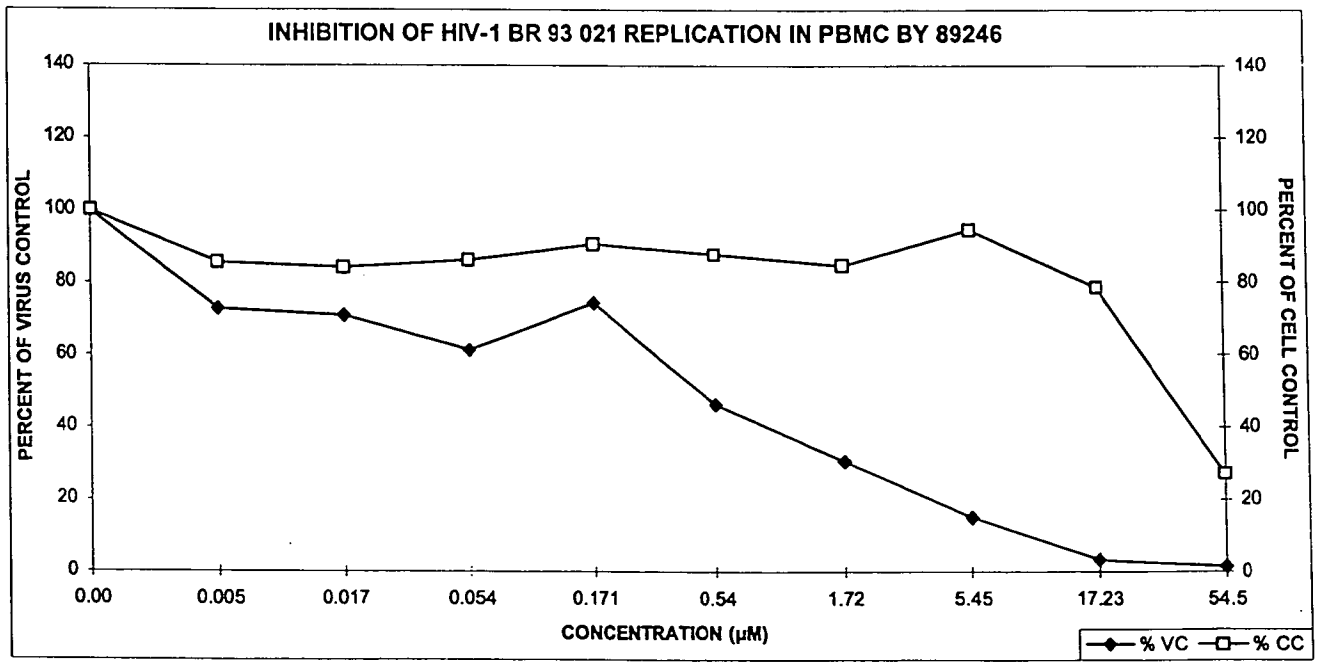
Virus/Strain: HIV-1 / ROJO
Virus Date/Titer: 12/16/02, 3.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 BR 93 021 REPLICATION IN PBMC BY 89246

RT Values(cpm)										
CONC (μM)	0.00	0.005	0.017	0.054	0.171	0.54	1.72	5.45	17.23	54.5
SAMPLE 1	16603	10006	10168	6240	8900	8383	4069	2095	366	146
SAMPLE 2	13824	10406	10828	10370	10741	6654	4852	2725	549	168
SAMPLE 3	13535	11613	10243	10370	13127	5384	4564	1787	571	308
MEAN	14653.8	10675.0	10413.0	8993.3	10922.7	6807.0	4495.0	2202.3	495.3	207.3
% VC	100.0	72.8	71.1	61.4	74.5	46.5	30.7	15.0	3.4	1.4

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.005	0.017	0.054	0.171	0.54	1.72	5.45	17.23	54.5
SAMPLE 1	0.838	0.703	0.679	0.747	0.846	0.759	0.696	1.001	0.859	0.238
SAMPLE 2	0.833	0.775	0.752	0.727	0.771	0.736	0.765	0.701	0.608	0.252
SAMPLE 3	0.922	0.741	0.754	0.765	0.736	0.784	0.741	0.752	0.575	0.214
MEAN	0.864	0.740	0.729	0.746	0.784	0.760	0.734	0.818	0.681	0.235
% CC	100.0	85.6	84.3	86.3	90.7	87.9	84.9	94.6	78.7	27.2

IC50 (μM) =0.47	TC50 (μM) =32.7	TI = 69.57



Client: IR&D
Investigator: Ptak
Setup Date: 04/02/04

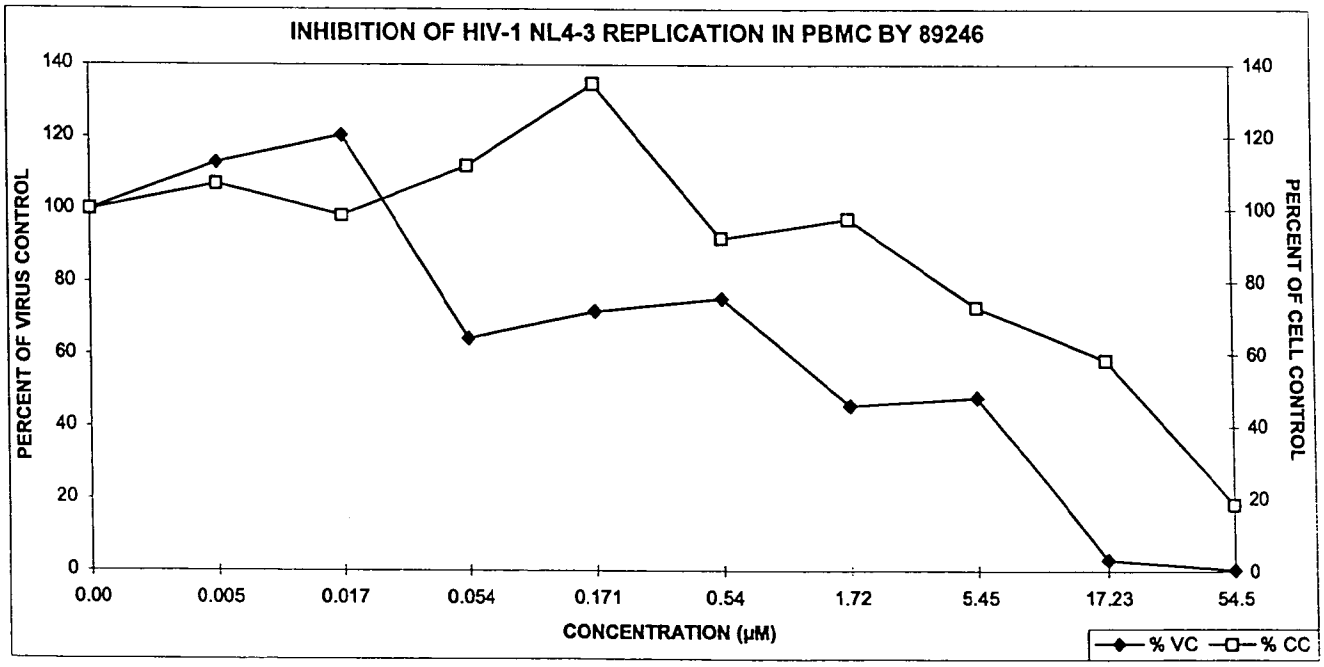
Virus/Strain: HIV-1 / BR 93 021
Virus Date/Titer: 10/18/99, 3.3 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY 89246

RT Values(cpm)										
CONC (µM)	0.00	0.005	0.017	0.054	0.171	0.54	1.72	5.45	17.23	54.5
SAMPLE 1	17547	34917	39963	28273	37278	18659	20590	22091	527	154
SAMPLE 2	39386	34080	45518	20288	13958	33731	9041	15355	395	139
SAMPLE 3	44311	45313	36429	16796	21620	24112	17009	11343	2292	198
MEAN	33747.8	38103.3	40636.7	21785.7	24285.3	25500.7	15546.7	16263.0	1071.3	163.7
% VC	100.0	112.9	120.4	64.6	72.0	75.6	46.1	48.2	3.2	0.5

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (µM)	0.00	0.005	0.017	0.054	0.171	0.54	1.72	5.45	17.23	54.5
SAMPLE 1	0.762	1.042	0.741	1.088	1.105	0.630	0.596	0.511	0.440	0.144
SAMPLE 2	0.806	0.733	0.839	0.847	1.042	0.716	0.821	0.520	0.420	0.129
SAMPLE 3	0.707	0.660	0.656	0.611	0.920	0.747	0.796	0.633	0.467	0.144
MEAN	0.758	0.812	0.745	0.849	1.022	0.698	0.738	0.555	0.442	0.139
% CC	100.0	107.1	98.3	111.9	134.8	92.0	97.3	73.1	58.3	18.3

IC50 (µM) =1.47	TC50 (µM) =21.9	TI = 14.90



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

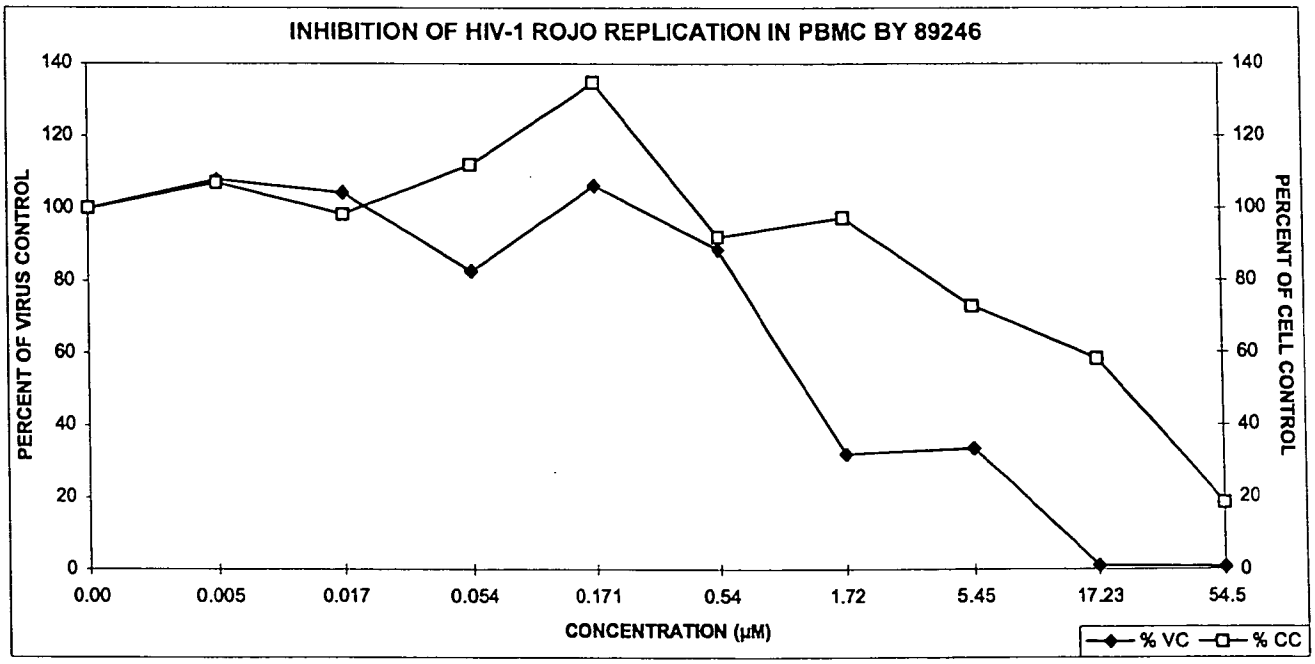
Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 4/25/03, 1.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 ROJO REPLICATION IN PBMC BY 89246

RT Values(cpm)										
CONC (μM)	0.00	0.005	0.017	0.054	0.171	0.54	1.72	5.45	17.23	54.5
SAMPLE 1	77999	44713	69109	40320	45219	60905	15458	27663	469	388
SAMPLE 2	36238	65740	56807	31933	57191	25175	22841	7913	996	322
SAMPLE 3	46860	63284	42018	60974	68532	56731	13240	18546	395	461
MEAN	53698.8	57912.3	55978.0	44409.0	56980.7	47603.7	17179.7	18040.7	620.0	390.3
% VC	100.0	107.8	104.2	82.7	106.1	88.6	32.0	33.6	1.2	0.7

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.005	0.017	0.054	0.171	0.54	1.72	5.45	17.23	54.5
SAMPLE 1	0.762	1.042	0.741	1.088	1.105	0.630	0.596	0.511	0.440	0.144
SAMPLE 2	0.806	0.733	0.839	0.847	1.042	0.716	0.821	0.520	0.420	0.129
SAMPLE 3	0.707	0.660	0.656	0.611	0.920	0.747	0.796	0.633	0.467	0.144
MEAN	0.758	0.812	0.745	0.849	1.022	0.698	0.738	0.555	0.442	0.139
% CC	100.0	107.1	98.3	111.9	134.8	92.0	97.3	73.1	58.3	18.3

IC50 (μM) =1.19	TC50 (μM) =21.9	TI = 18.40



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

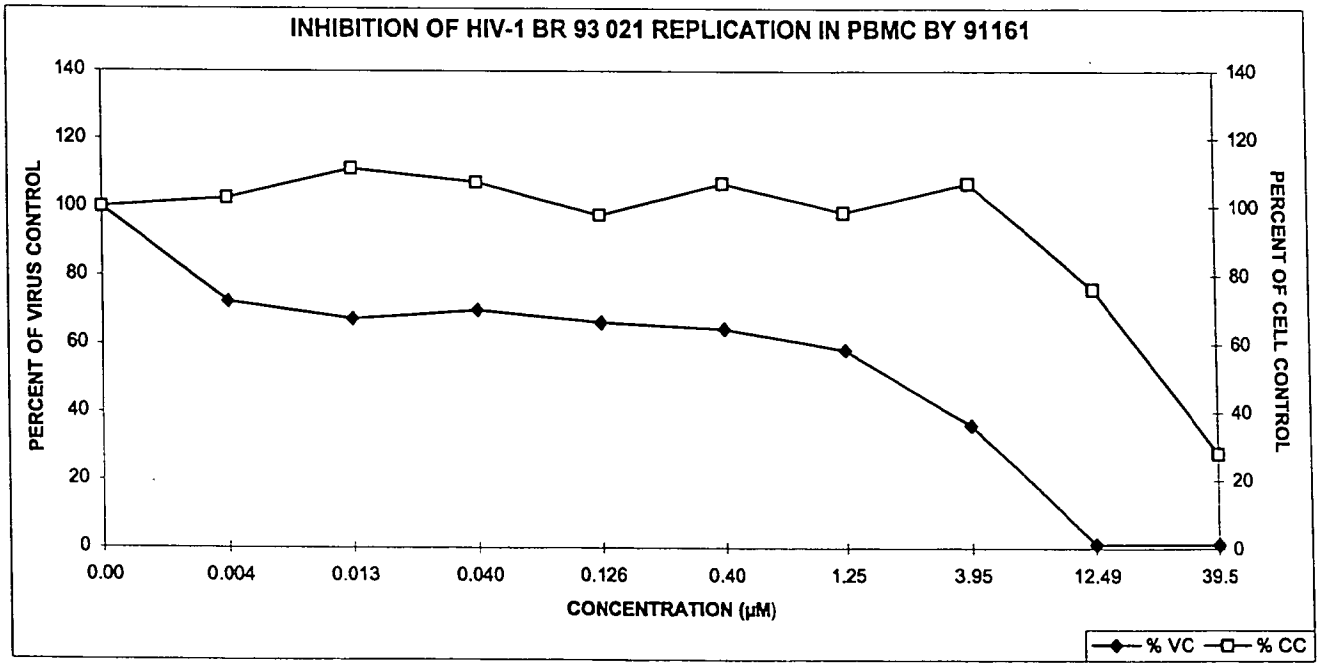
Virus/Strain: HIV-1 / ROJO
Virus Date/Titer: 12/16/02, 3.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 BR 93 021 REPLICATION IN PBMC BY 91161

RT Values(cpm)										
CONC (µM)	0.00	0.004	0.013	0.040	0.126	0.40	1.25	3.95	12.49	39.5
SAMPLE 1	13398	9908	11039	10454	10139	10410	9143	6190	195	202
SAMPLE 2	11871	8836	6907	9246	6243	6514	6331	3552	115	144
SAMPLE 3	12162	8392	7291	6507	8440	7231	6411	3833	158	137
MEAN	12476.7	9045.3	8412.3	8735.7	8274.0	8051.7	7295.0	4525.0	156.0	161.0
% VC	100.0	72.5	67.4	70.0	66.3	64.5	58.5	36.3	1.3	1.3

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (µM)	0.00	0.004	0.013	0.040	0.126	0.40	1.25	3.95	12.49	39.5
SAMPLE 1	0.846	0.814	0.951	0.920	0.816	0.871	0.944	0.996	0.669	0.231
SAMPLE 2	0.823	0.827	0.872	0.841	0.727	1.001	0.779	0.798	0.598	0.217
SAMPLE 3	0.829	0.921	0.955	0.917	0.895	0.797	0.735	0.877	0.631	0.248
MEAN	0.832	0.854	0.926	0.893	0.813	0.890	0.819	0.890	0.633	0.232
% CC	100.0	102.6	111.2	107.2	97.6	106.9	98.4	106.9	76.0	27.9

IC50 (µM) =1.94	TC50 (µM) =23.3	TI = 12.01



Client: IR&D
Investigator: Ptak
Setup Date: 04/02/04

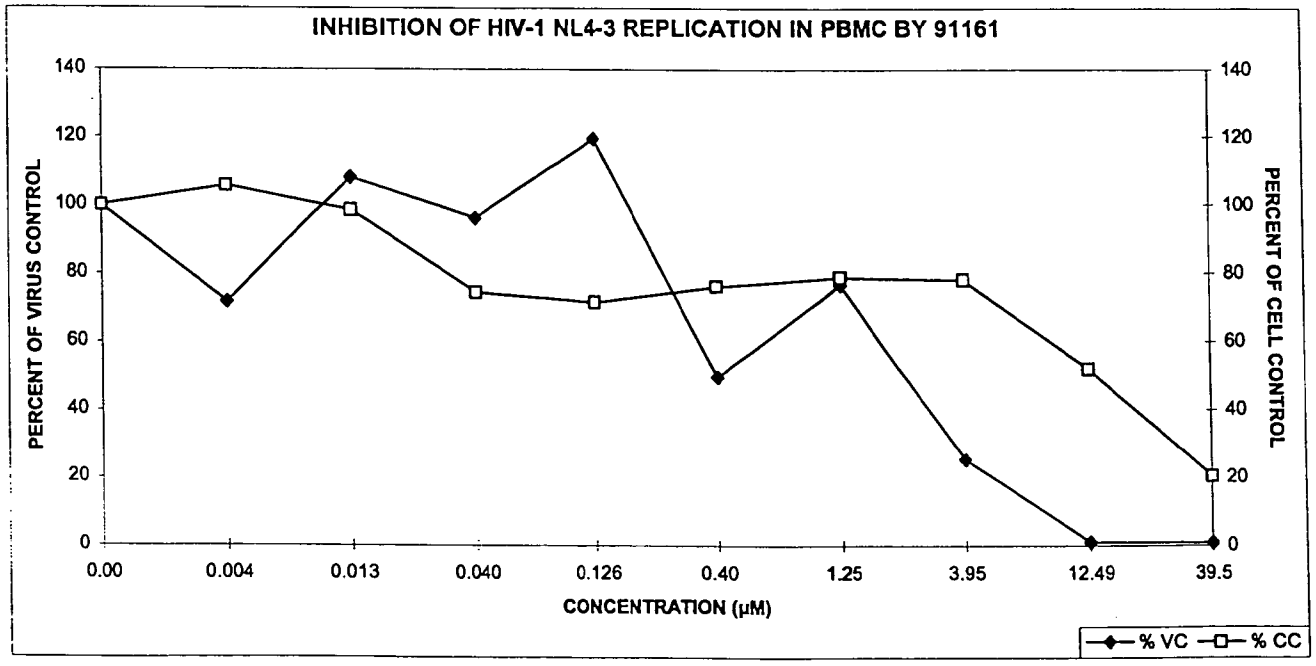
Virus/Strain: HIV-1 / BR 93 021
Virus Date/Titer: 10/18/99, 3.3 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY 91161

RT Values(cpm)										
CONC (μM)	0.00	0.004	0.013	0.040	0.126	0.40	1.25	3.95	12.49	39.5
SAMPLE 1	44169	29258	37384	36242	51638	14616	22142	4165	180	252
SAMPLE 2	16907	21839	37229	26479	36550	17554	23140	6587	245	288
SAMPLE 3	37177	19560	31611	31893	29214	16872	30300	14284	649	259
MEAN	32750.7	23552.3	35408.0	31538.0	39134.0	16347.3	25194.0	8345.3	358.0	266.3
% VC	100.0	71.9	108.1	96.3	119.5	49.9	76.9	25.5	1.1	0.8

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.004	0.013	0.040	0.126	0.40	1.25	3.95	12.49	39.5
SAMPLE 1	0.618	0.646	0.660	0.546	0.485	0.526	0.515	0.512	0.347	0.148
SAMPLE 2	0.725	0.889	0.764	0.514	0.527	0.558	0.572	0.533	0.344	0.123
SAMPLE 3	0.785	0.717	0.674	0.530	0.518	0.545	0.599	0.624	0.417	0.165
MEAN	0.710	0.750	0.699	0.530	0.510	0.543	0.562	0.556	0.369	0.145
% CC	100.0	105.7	98.5	74.7	71.8	76.5	79.2	78.4	52.0	20.5

IC50 (μM) =2.28	TC50 (μM) =13.5	TI = 5.92



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

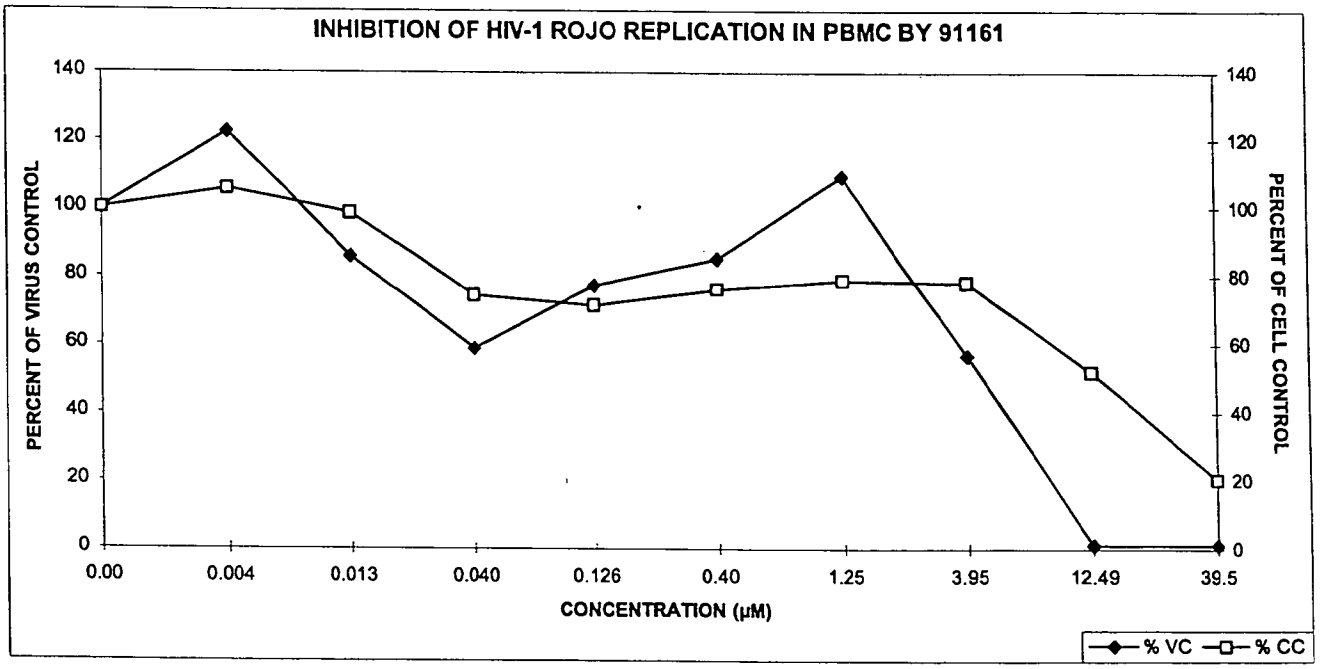
Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 4/25/03, 1.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 ROJO REPLICATION IN PBMC BY 91161

RT Values(cpm)										
CONC (μM)	0.00	0.004	0.013	0.040	0.126	0.40	1.25	3.95	12.49	39.5
SAMPLE 1	58850	64169	17969	16945	11304	43213	45831	25558	771	720
SAMPLE 2	14850	80121	47356	24198	45257	25316	63896	21509	555	403
SAMPLE 3	73048	35305	60845	45343	57268	56924	50950	36453	555	562
MEAN	48915.7	59865.0	42056.7	28828.7	37943.0	41817.7	53559.0	27840.0	627.0	561.7
% VC	100.0	122.4	86.0	58.9	77.6	85.5	109.5	56.9	1.3	1.1

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.004	0.013	0.040	0.126	0.40	1.25	3.95	12.49	39.5
SAMPLE 1	0.618	0.646	0.660	0.546	0.485	0.526	0.515	0.512	0.347	0.148
SAMPLE 2	0.725	0.889	0.764	0.514	0.527	0.558	0.572	0.533	0.344	0.123
SAMPLE 3	0.785	0.717	0.674	0.530	0.518	0.545	0.599	0.624	0.417	0.165
MEAN	0.710	0.750	0.699	0.530	0.510	0.543	0.562	0.556	0.369	0.145
% CC	100.0	105.7	98.5	74.7	71.8	76.5	79.2	78.4	52.0	20.5

IC50 (μM) =4.56	TC50 (μM) =13.5	TI = 2.96



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

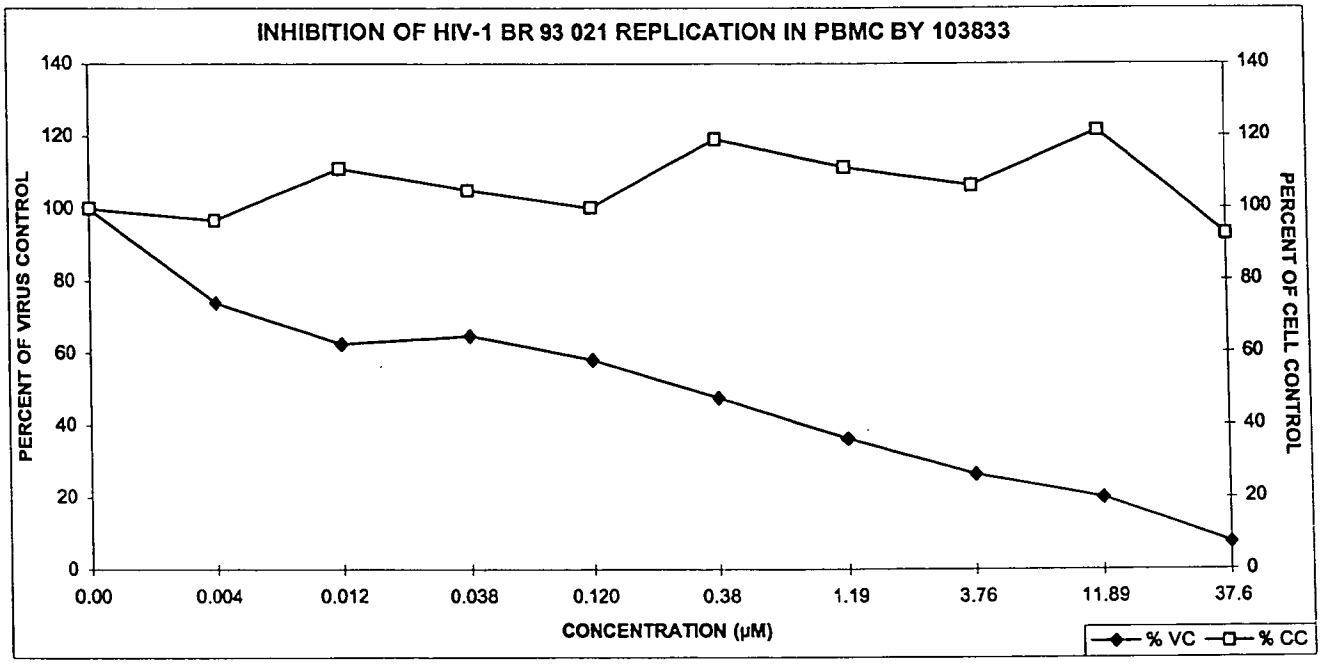
Virus/Strain: HIV-1 / ROJO
Virus Date/Titer: 12/16/02, 3.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 BR 93 021 REPLICATION IN PBMC BY 103833

RT Values(cpm)										
CONC (μM)	0.00	0.004	0.012	0.038	0.120	0.38	1.19	3.76	11.89	37.6
SAMPLE 1	13398	8347	7170	6824	6720	6861	3677	1714	2608	835
SAMPLE 2	11871	8910	5867	6809	6159	4888	5265	3802	2307	710
SAMPLE 3	12162	10435	10405	10629	8923	6078	4659	4395	2564	1274
MEAN	12476.7	9230.7	7814.0	8087.3	7267.3	5942.3	4533.7	3303.7	2493.0	939.7
% VC	100.0	74.0	62.6	64.8	58.2	47.6	36.3	26.5	20.0	7.5

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.004	0.012	0.038	0.120	0.38	1.19	3.76	11.89	37.6
SAMPLE 1	0.846	0.747	0.888	0.839	0.820	1.132	0.935	0.822	0.896	0.844
SAMPLE 2	0.823	0.858	0.938	0.878	0.774	0.799	0.856	0.876	1.017	0.706
SAMPLE 3	0.829	0.809	0.942	0.904	0.906	1.043	0.988	0.953	1.117	0.762
MEAN	0.832	0.805	0.923	0.874	0.833	0.991	0.926	0.884	1.010	0.771
% CC	100.0	96.7	110.9	104.9	100.1	119.1	111.3	106.2	121.4	92.6

IC50 (μM) =0.29	TC50 (μM) =>37.6	TI = >129.66



Client: IR&D
Investigator: Ptak
Setup Date: 04/02/04

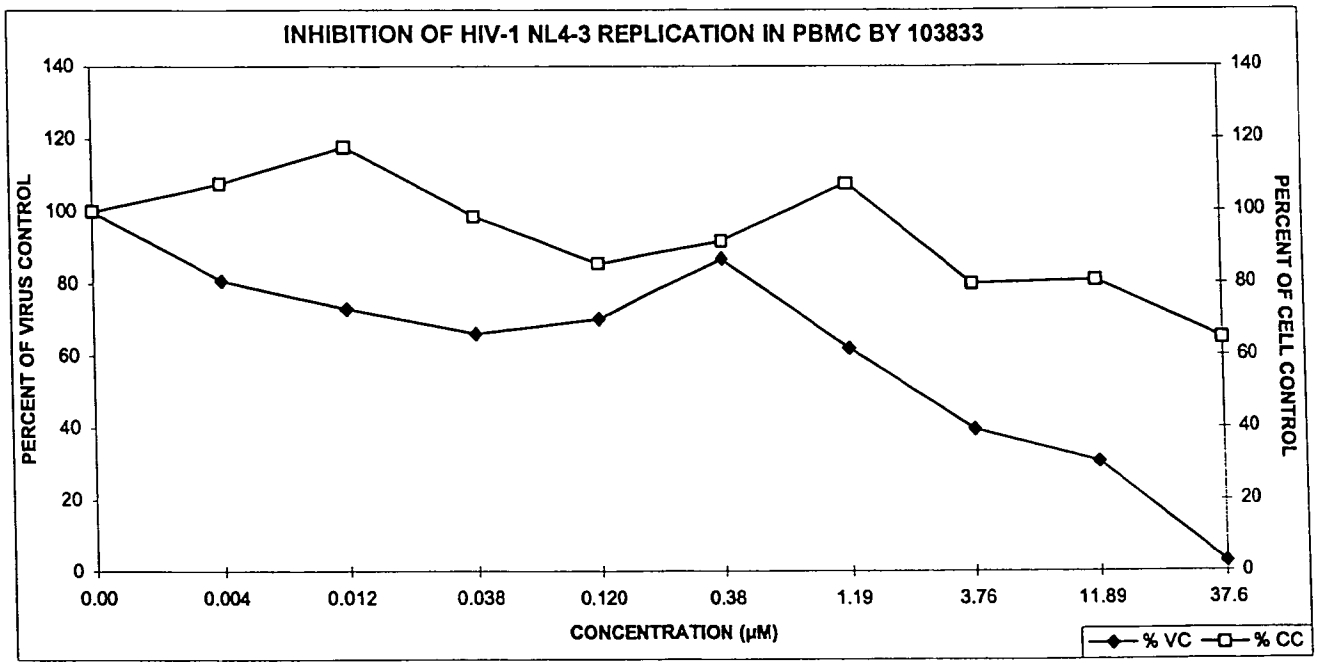
Virus/Strain: HIV-1 / BR 93 021
Virus Date/Titer: 10/18/99, 3.3 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY 103833

RT Values(cpm)										
CONC (μM)	0.00	0.004	0.012	0.038	0.120	0.38	1.19	3.76	11.89	37.6
SAMPLE 1	44169	26982	26855	12877	24304	19361	18873	14842	8895	996
SAMPLE 2	16907	22134	22682	21255	13765	27178	21093	11879	5766	1091
SAMPLE 3	37177	30344	22207	30888	30975	38826	21056	12282	15443	549
MEAN	32750.7	26486.7	23914.7	21673.3	23014.7	28455.0	20340.7	13001.0	10034.7	878.7
% VC	100.0	80.9	73.0	66.2	70.3	86.9	62.1	39.7	30.6	2.7

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.004	0.012	0.038	0.120	0.38	1.19	3.76	11.89	37.6
SAMPLE 1	0.618	0.536	0.698	0.624	0.584	0.584	0.724	0.527	0.604	0.451
SAMPLE 2	0.725	0.874	0.883	0.567	0.526	0.702	0.722	0.559	0.561	0.501
SAMPLE 3	0.785	0.879	0.923	0.904	0.710	0.666	0.843	0.614	0.555	0.430
MEAN	0.710	0.763	0.835	0.698	0.607	0.651	0.763	0.567	0.573	0.461
% CC	100.0	107.6	117.6	98.4	85.5	91.7	107.5	79.9	80.8	64.9

IC50 (μM) =2.22	TC50 (μM) =>37.6	TI = >16.94



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

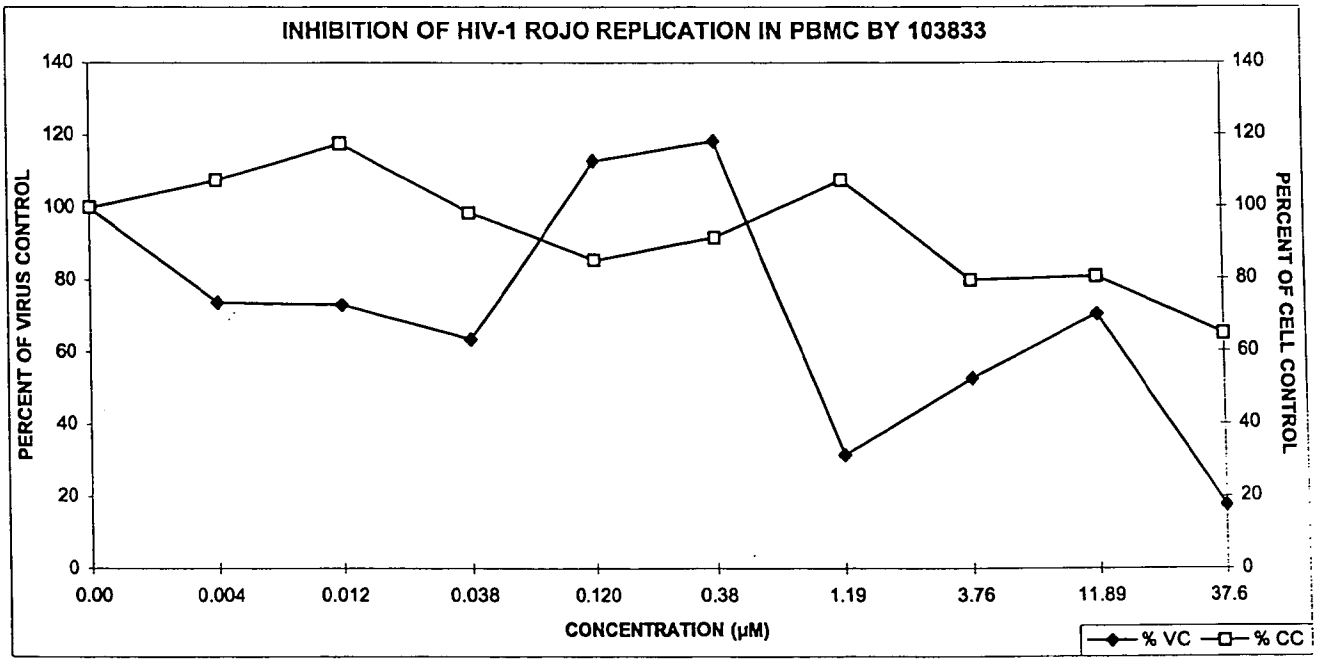
Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 4/25/03, 1.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 ROJO REPLICATION IN PBMC BY 103833

RT Values(cpm)										
CONC (µM)	0.00	0.004	0.012	0.038	0.120	0.38	1.19	3.76	11.89	37.6
SAMPLE 1	58850	27193	18140	56582	48729	48652	1816	20602	32877	1158
SAMPLE 2	14850	37159	73934	25143	58763	71362	4491	49392	26990	9096
SAMPLE 3	73048	44112	15178	11761	58032	53533	39956	7547	43475	15666
MEAN	48915.7	36154.7	35750.7	31162.0	55174.7	57849.0	15421.0	25847.0	34447.3	8640.0
% VC	100.0	73.9	73.1	63.7	112.8	118.3	31.5	52.8	70.4	17.7

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (µM)	0.00	0.004	0.012	0.038	0.120	0.38	1.19	3.76	11.89	37.6
SAMPLE 1	0.618	0.536	0.698	0.624	0.584	0.584	0.724	0.527	0.604	0.451
SAMPLE 2	0.725	0.874	0.883	0.567	0.526	0.702	0.722	0.559	0.561	0.501
SAMPLE 3	0.785	0.879	0.923	0.904	0.710	0.666	0.843	0.614	0.555	0.430
MEAN	0.710	0.763	0.835	0.698	0.607	0.651	0.763	0.567	0.573	0.461
% CC	100.0	107.6	117.6	98.4	85.5	91.7	107.5	79.9	80.8	64.9

IC50 (µM) =18.6	TC50 (µM) =>37.6	TI = >2.02



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

Virus/Strain: HIV-1 / ROJO
Virus Date/Titer: 12/16/02, 3.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 BR 93 021 REPLICATION IN PBMC BY 104366

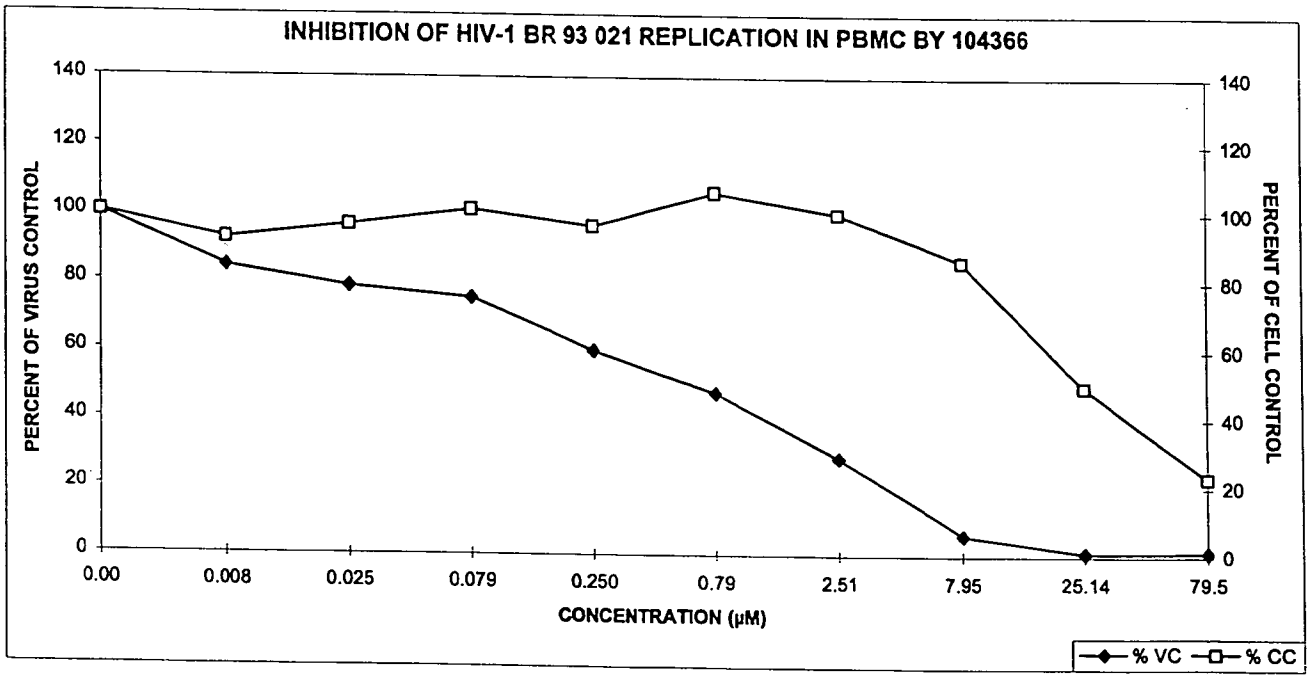
RT Values(cpm)										
CONC (μM)	0.00	0.008	0.025	0.079	0.250	0.79	2.51	7.95	25.14	79.5
SAMPLE 1	16149	15039	14876	13310	11187	10425	4091	893	166	223
SAMPLE 2	14353	12894	10380	13193	8850	7151	5767	1412	195	137
SAMPLE 3	16813	11985	11927	9172	8367	5020	3730	584	166	216
MEAN	15771.2	13306.0	12394.3	11891.7	9468.0	7532.0	4529.3	963.0	175.7	192.0
% VC	100.0	84.4	78.6	75.4	60.0	47.8	28.7	6.1	1.1	1.2

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.008	0.025	0.079	0.250	0.79	2.51	7.95	25.14	79.5
SAMPLE 1	0.756	0.782	0.789	0.778	0.808	0.742	0.740	0.684	0.359	0.183
SAMPLE 2	0.795	0.677	0.676	0.740	0.746	0.825	0.743	0.697	0.365	0.181
SAMPLE 3	0.874	0.782	0.876	0.932	0.780	1.009	0.943	0.712	0.480	0.194
MEAN	0.808	0.747	0.780	0.816	0.778	0.859	0.809	0.698	0.401	0.186
% CC	100.0	92.4	96.5	101.0	96.3	106.2	100.0	86.3	49.6	23.0

IC50 (μM) =0.64

TC50 (μM) =24.9

TI = 38.91



Client: IR&D
Investigator: Ptak
Setup Date: 04/02/04

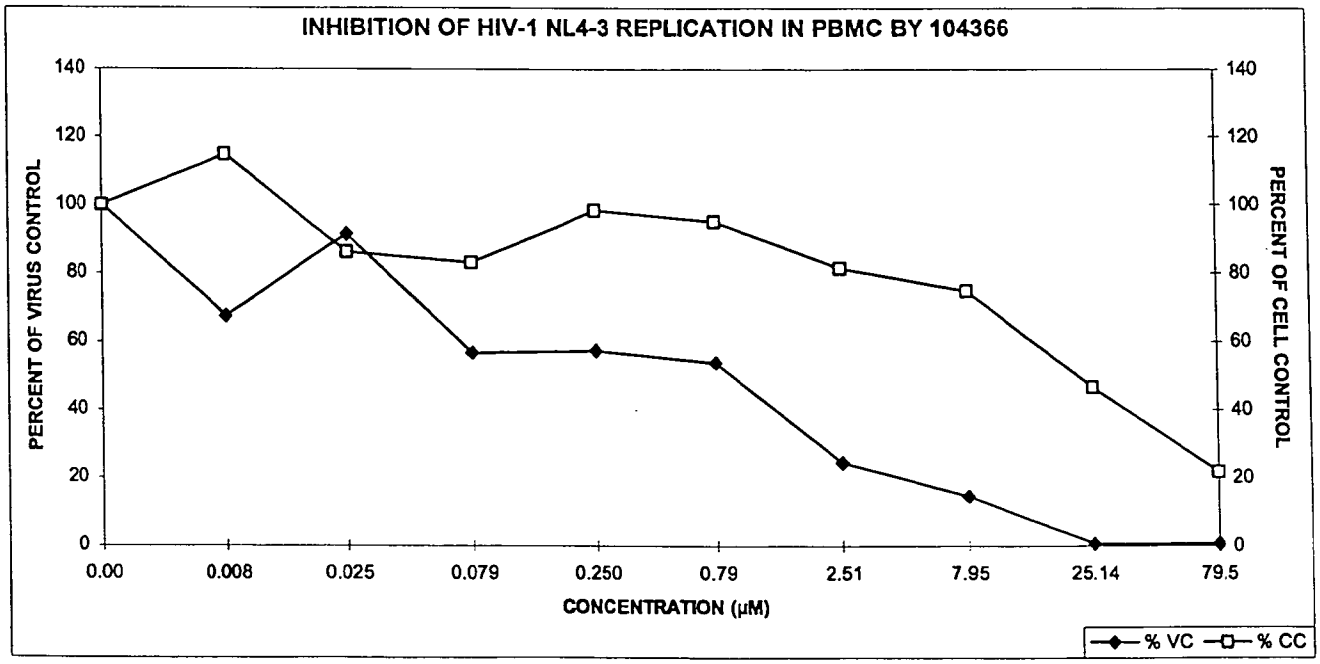
Virus/Strain: HIV-1 / BR 93 021
Virus Date/Titer: 10/18/99, 3.3 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY 104366

RT Values(cpm)										
CONC (μM)	0.00	0.008	0.025	0.079	0.250	0.79	2.51	7.95	25.14	79.5
SAMPLE 1	26269	9450	24856	16432	8016	11633	3600	4410	231	209
SAMPLE 2	19305	22756	25716	18712	26337	11282	1529	1102	166	151
SAMPLE 3	28987	18219	17753	7239	8484	17348	13134	5420	173	130
MEAN	24853.5	16808.3	22775.0	14127.7	14279.0	13421.0	6087.7	3644.0	190.0	163.3
% VC	100.0	67.6	91.6	56.8	57.5	54.0	24.5	14.7	0.8	0.7

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.008	0.025	0.079	0.250	0.79	2.51	7.95	25.14	79.5
SAMPLE 1	0.671	0.673	0.560	0.500	0.637	0.552	0.550	0.482	0.253	0.147
SAMPLE 2	0.601	0.848	0.584	0.593	0.594	0.805	0.544	0.479	0.338	0.136
SAMPLE 3	0.683	0.727	0.545	0.534	0.695	0.506	0.502	0.504	0.322	0.142
MEAN	0.652	0.749	0.563	0.542	0.642	0.621	0.532	0.489	0.304	0.142
% CC	100.0	114.9	86.4	83.2	98.5	95.2	81.6	75.0	46.7	21.7

IC50 (μM) =0.92	TC50 (μM) =22.0	TI = 23.91



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

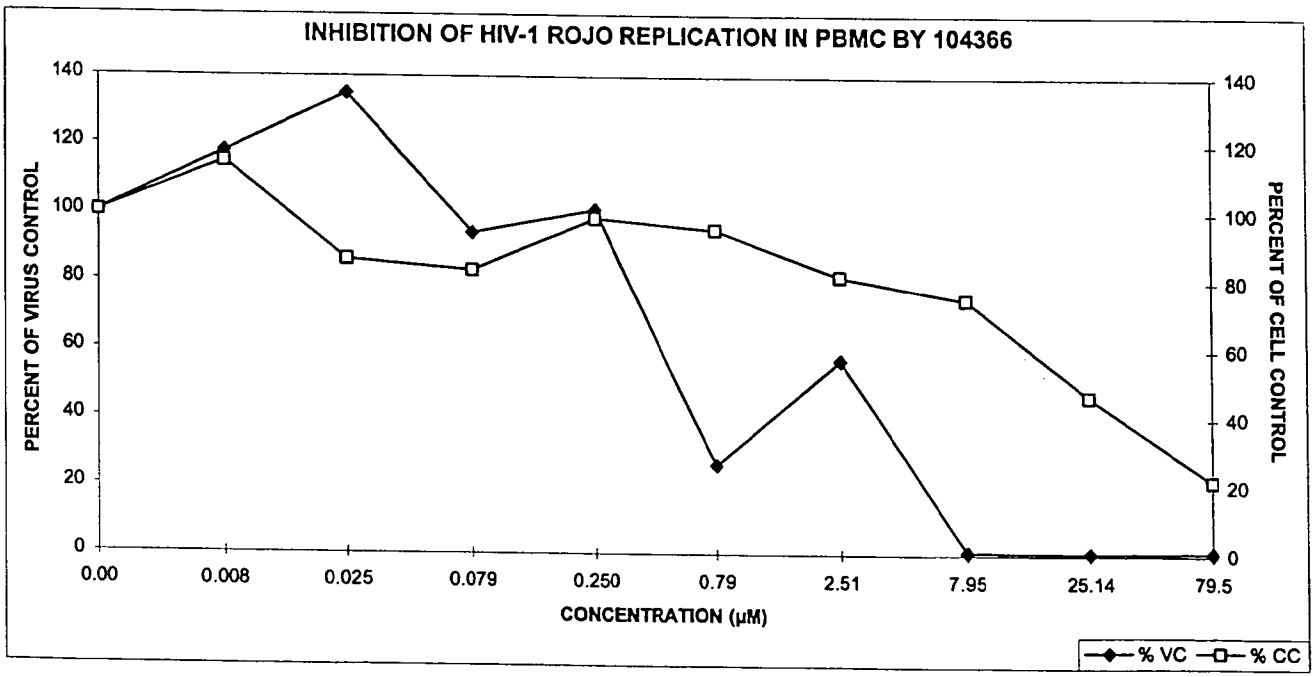
Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 4/25/03, 1.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 ROJO REPLICATION IN PBMC BY 104366

RT Values(cpm)										
CONC (μM)	0.00	0.008	0.025	0.079	0.250	0.79	2.51	7.95	25.14	79.5
SAMPLE 1	62618	90093	32425	49677	76881	3015	22070	555	331	324
SAMPLE 2	26277	24278	67558	47184	1786	27065	26199	360	303	540
SAMPLE 3	47188	45835	83623	31207	58695	5819	29463	303	382	403
MEAN	45360.8	53402.0	61202.0	42689.3	45787.3	11966.3	25910.7	406.0	338.7	422.3
% VC	100.0	117.7	134.9	94.1	100.9	26.4	57.1	0.9	0.7	0.9

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.008	0.025	0.079	0.250	0.79	2.51	7.95	25.14	79.5
SAMPLE 1	0.671	0.673	0.560	0.500	0.637	0.552	0.550	0.482	0.253	0.147
SAMPLE 2	0.601	0.848	0.584	0.593	0.594	0.805	0.544	0.479	0.338	0.136
SAMPLE 3	0.683	0.727	0.545	0.534	0.695	0.506	0.502	0.504	0.322	0.142
MEAN	0.652	0.749	0.563	0.542	0.642	0.621	0.532	0.489	0.304	0.142
% CC	100.0	114.9	86.4	83.2	98.5	95.2	81.6	75.0	46.7	21.7

IC50 (μM) =2.90	TC50 (μM) =22.0	TI = 7.59



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

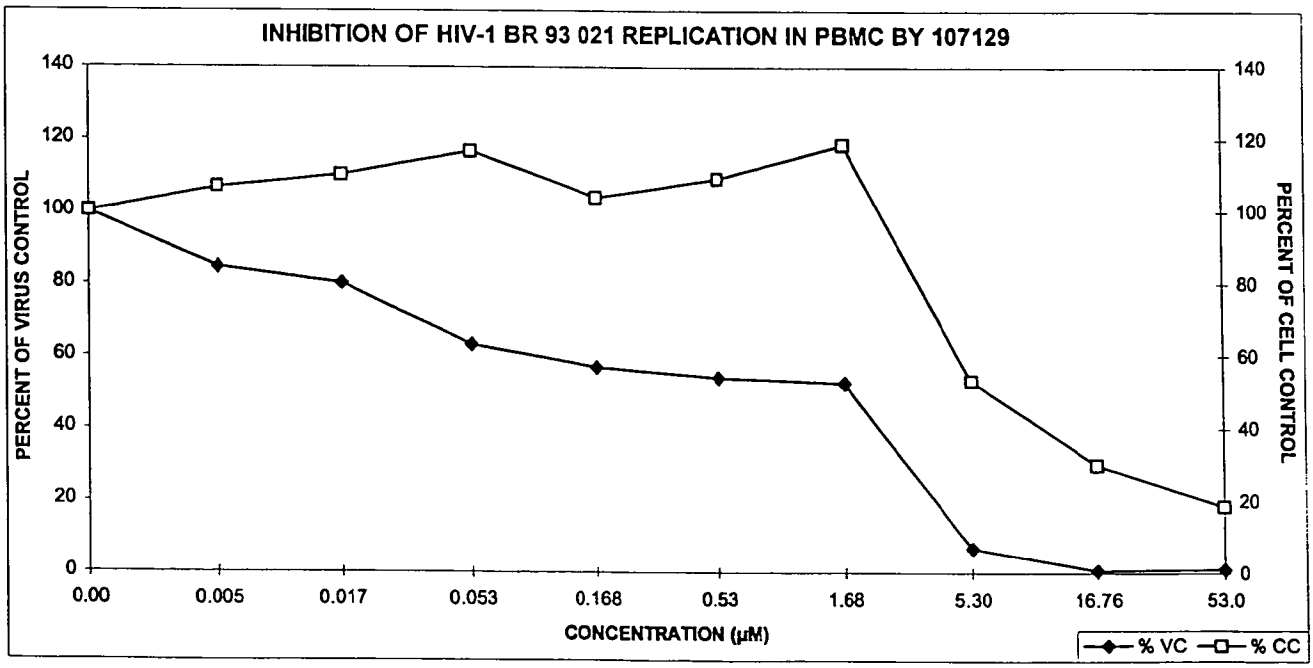
Virus/Strain: HIV-1 / ROJO
Virus Date/Titer: 12/16/02, 3.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 BR 93 021 REPLICATION IN PBMC BY 107129

RT Values(cpm)										
CONC (µM)	0.00	0.005	0.017	0.053	0.168	0.53	1.68	5.30	16.76	53.0
SAMPLE 1	16149	12515	11360	9299	9410	7341	9195	2263	59	161
SAMPLE 2	14353	12686	13730	10363	8332	8353	6698	168	190	183
SAMPLE 3	16813	14841	12886	10274	9210	9920	9099	769	139	146
MEAN	15771.2	13347.3	12658.7	9978.7	8984.0	8538.0	8330.7	1066.7	129.3	163.3
% VC	100.0	84.6	80.3	63.3	57.0	54.1	52.8	6.8	0.8	1.0

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (µM)	0.00	0.005	0.017	0.053	0.168	0.53	1.68	5.30	16.76	53.0
SAMPLE 1	0.756	0.748	0.785	0.808	0.744	0.831	0.939	0.618	0.277	0.147
SAMPLE 2	0.795	0.942	0.884	1.015	0.851	0.897	0.874	0.162	0.223	0.158
SAMPLE 3	0.874	0.900	1.000	1.007	0.922	0.917	1.064	0.515	0.227	0.144
MEAN	0.808	0.863	0.890	0.943	0.839	0.882	0.959	0.431	0.242	0.150
% CC	100.0	106.8	110.0	116.7	103.8	109.1	118.6	53.4	30.0	18.5

IC50 (µM) =1.80	TC50 (µM) =6.26	TI = 3.48



Client: IR&D
Investigator: Ptak
Setup Date: 04/02/04

Virus/Strain: HIV-1 / BR 93 021
Virus Date/Titer: 10/18/99, 3.3 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY 107129

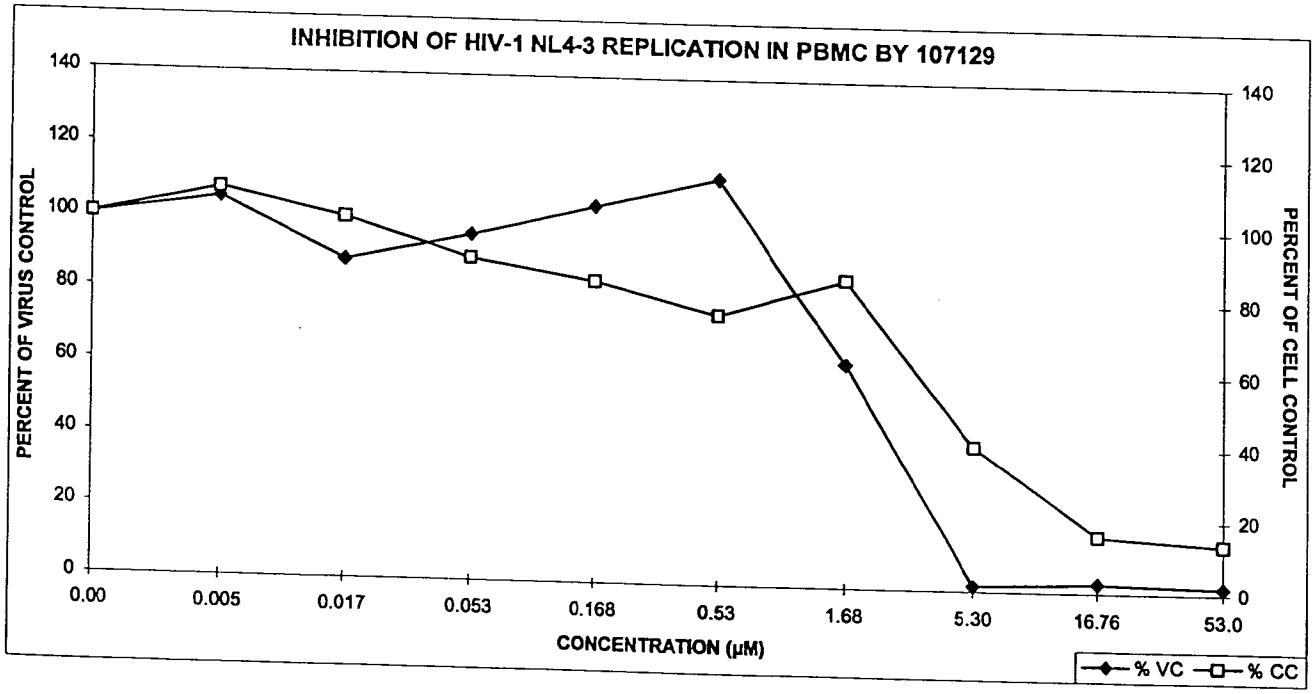
RT Values(cpm)										
CONC (μM)	0.00	0.005	0.017	0.053	0.168	0.53	1.68	5.30	16.76	53.0
SAMPLE 1	26269	37339	20924	27604	26739	27567	17378	593	411	718
SAMPLE 2	19305	24248	21495	20205	32198	20996	761	264	1246	293
SAMPLE 3	28987	16656	23437	23667	18865	35305	28414	271	286	278
MEAN	24853.5	26081.0	21952.0	23825.3	25934.0	27956.0	15517.7	376.0	647.7	429.7
% VC	100.0	104.9	88.3	95.9	104.3	112.5	62.4	1.5	2.6	1.7

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.005	0.017	0.053	0.168	0.53	1.68	5.30	16.76	53.0
SAMPLE 1	0.671	0.664	0.686	0.517	0.606	0.544	0.458	0.307	0.107	0.084
SAMPLE 2	0.601	0.620	0.557	0.483	0.506	0.403	0.519	0.247	0.095	0.093
SAMPLE 3	0.683	0.820	0.714	0.747	0.526	0.520	0.695	0.232	0.104	0.087
MEAN	0.652	0.701	0.652	0.583	0.546	0.489	0.557	0.262	0.102	0.088
% CC	100.0	107.6	100.0	89.4	83.8	75.0	85.5	40.2	15.6	13.5

IC50 (μM) =2.12

TC50 (μM) =4.13

TI = 1.95



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

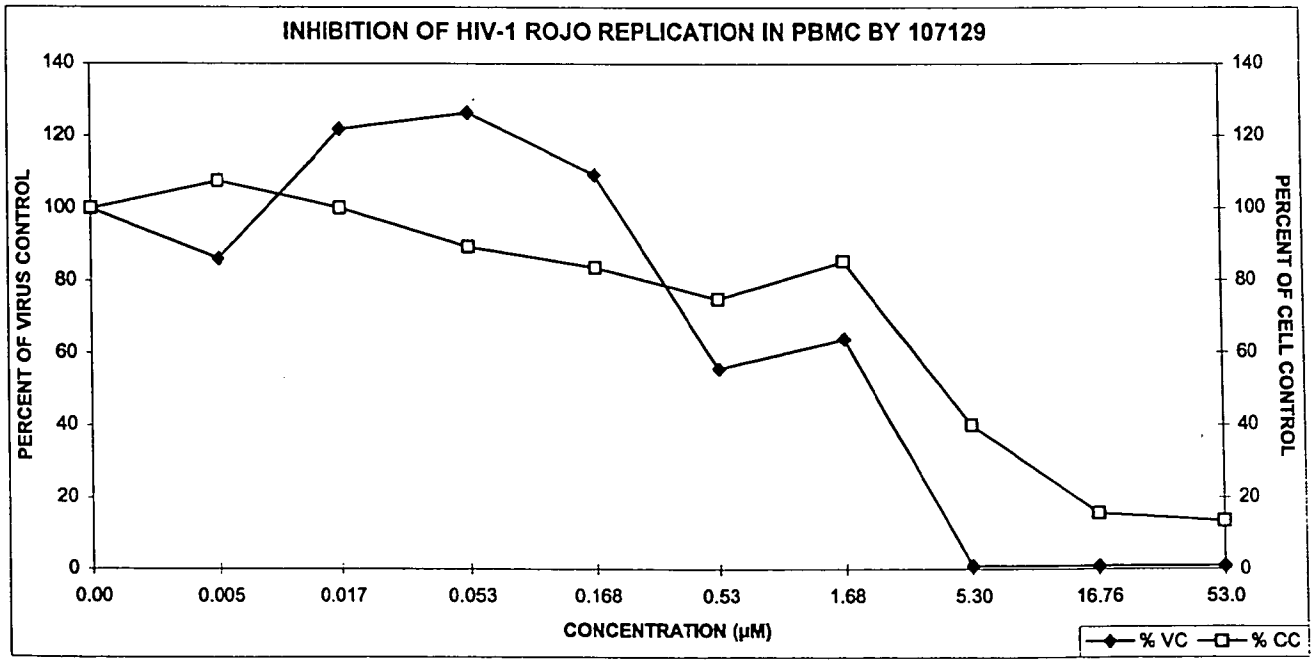
Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 4/25/03, 1.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 ROJO REPLICATION IN PBMC BY 107129

RT Values(cpm)										
CONC (μM)	0.00	0.005	0.017	0.053	0.168	0.53	1.68	5.30	16.76	53.0
SAMPLE 1	62618	93379	59371	66180	33168	6906	43350	447	352	315
SAMPLE 2	26277	5883	40351	15645	65097	19526	43462	388	410	593
SAMPLE 3	47188	17880	65949	90208	50424	49521	384	359	469	308
MEAN	45360.8	39047.3	55223.7	57344.3	49563.0	25317.7	29065.3	398.0	410.3	405.3
% VC	100.0	86.1	121.7	126.4	109.3	55.8	64.1	0.9	0.9	0.9

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.005	0.017	0.053	0.168	0.53	1.68	5.30	16.76	53.0
SAMPLE 1	0.671	0.664	0.686	0.517	0.606	0.544	0.458	0.307	0.107	0.084
SAMPLE 2	0.601	0.620	0.557	0.483	0.506	0.403	0.519	0.247	0.095	0.093
SAMPLE 3	0.683	0.820	0.714	0.747	0.526	0.520	0.695	0.232	0.104	0.087
MEAN	0.652	0.701	0.652	0.583	0.546	0.489	0.557	0.262	0.102	0.088
% CC	100.0	107.6	100.0	89.4	83.8	75.0	85.5	40.2	15.6	13.5

IC50 (μM) =2.17	TC50 (μM) =4.13	TI = 1.90



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

Virus/Strain: HIV-1 / ROJO
Virus Date/Titer: 12/16/02, 3.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 BR 93 021 REPLICATION IN PBMC BY 107740

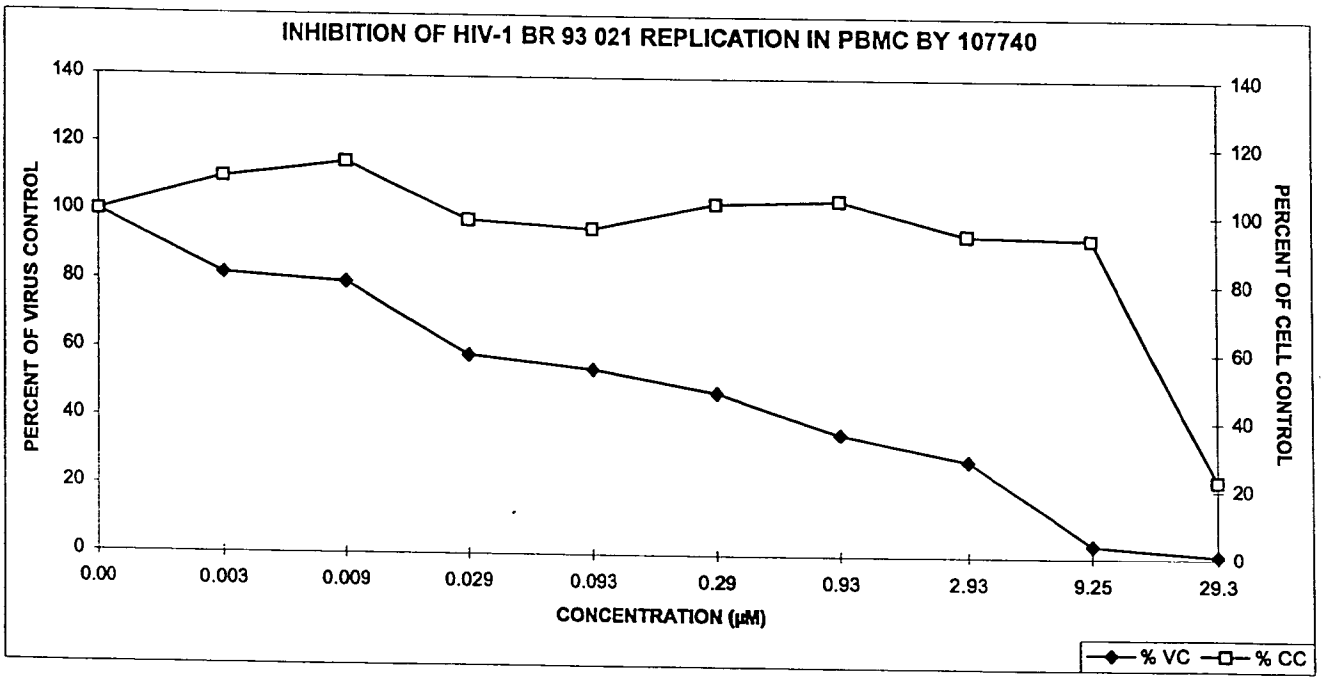
RT Values(cpm)										
CONC (μM)	0.00	0.003	0.009	0.029	0.093	0.29	0.93	2.93	9.25	29.3
SAMPLE 1	15509	15276	15505	9340	9993	7766	5540	4850	346	144
SAMPLE 2	12428	12141	10840	7898	7524	7019	5885	3495	699	144
SAMPLE 3	16750	9272	9309	8960	6858	6609	4626	4251	670	108
MEAN	14895.3	12229.7	11884.7	8732.7	8125.0	7131.3	5350.3	4198.7	571.7	132.0
% VC	100.0	82.1	79.8	58.6	54.5	47.9	35.9	28.2	3.8	0.9

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.003	0.009	0.029	0.093	0.29	0.93	2.93	9.25	29.3
SAMPLE 1	0.983	1.012	0.943	0.903	0.926	0.878	0.859	0.964	0.725	0.194
SAMPLE 2	0.858	0.923	1.239	0.790	0.788	0.924	0.863	0.758	0.841	0.195
SAMPLE 3	0.805	0.981	0.854	0.898	0.814	0.925	1.035	0.771	0.903	0.213
MEAN	0.882	0.972	1.012	0.863	0.843	0.909	0.919	0.831	0.823	0.201
% CC	100.0	110.2	114.7	97.9	95.6	103.1	104.2	94.2	93.3	22.8

IC50 (μM) =0.20

TC50 (μM) =18.8

TI = 94.00



Client: IR&D
Investigator: Ptak
Setup Date: 04/02/04

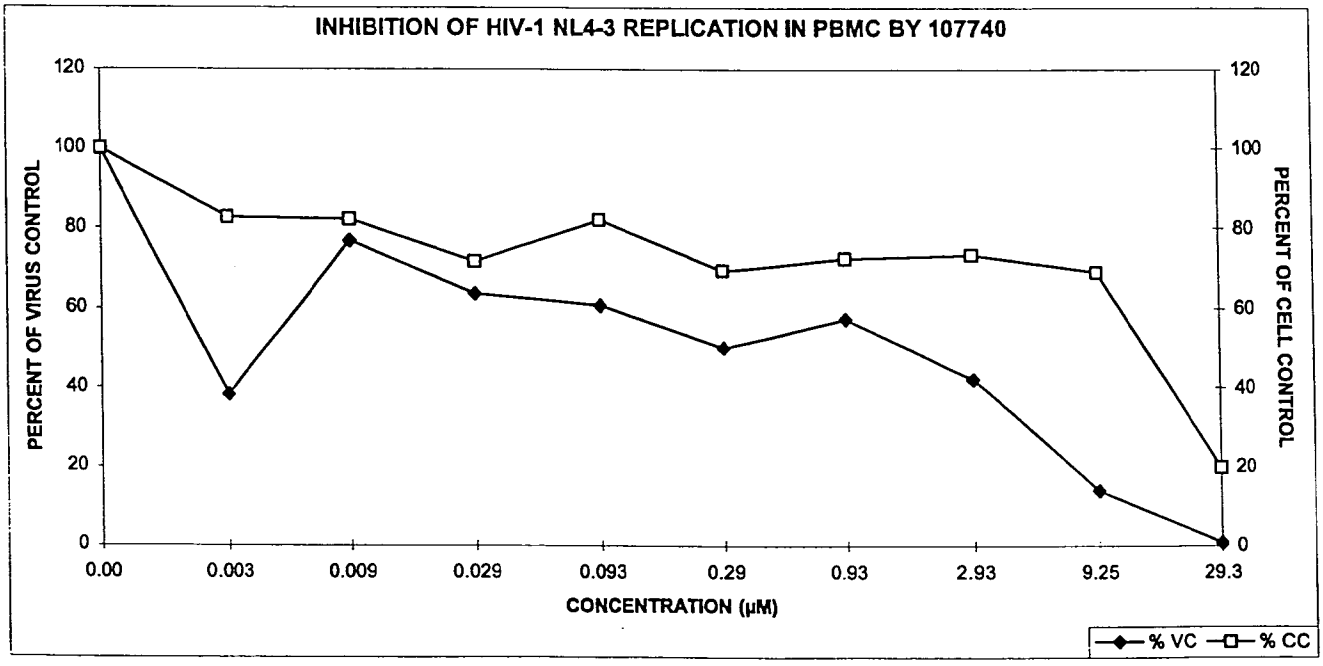
Virus/Strain: HIV-1 / BR 93 021
Virus Date/Titer: 10/18/99, 3.3 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY 107740

RT Values(cpm)										
CONC (μM)	0.00	0.003	0.009	0.029	0.093	0.29	0.93	2.93	9.25	29.3
SAMPLE 1	43455	8104	23939	23764	24652	7906	28223	18063	9327	187
SAMPLE 2	29494	27561	15698	26978	22159	7634	22862	22604	3343	252
SAMPLE 3	40549	7720	47620	21485	22032	41261	13955	7085	3106	403
MEAN	37832.3	14461.7	29085.7	24075.7	22947.7	18933.7	21680.0	15917.3	5258.7	280.7
% VC	100.0	38.2	76.9	63.6	60.7	50.0	57.3	42.1	13.9	0.7

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.003	0.009	0.029	0.093	0.29	0.93	2.93	9.25	29.3
SAMPLE 1	0.628	0.480	0.495	0.459	0.556	0.483	0.514	0.538	0.482	0.120
SAMPLE 2	0.705	0.623	0.602	0.479	0.556	0.465	0.507	0.479	0.480	0.142
SAMPLE 3	0.703	0.580	0.577	0.521	0.557	0.464	0.454	0.477	0.443	0.142
MEAN	0.679	0.561	0.558	0.486	0.557	0.471	0.492	0.498	0.468	0.134
% CC	100.0	82.7	82.2	71.6	82.0	69.4	72.5	73.4	69.0	19.8

IC50 (μM) =1.61	TC50 (μM) =14.4	TI = 8.94



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

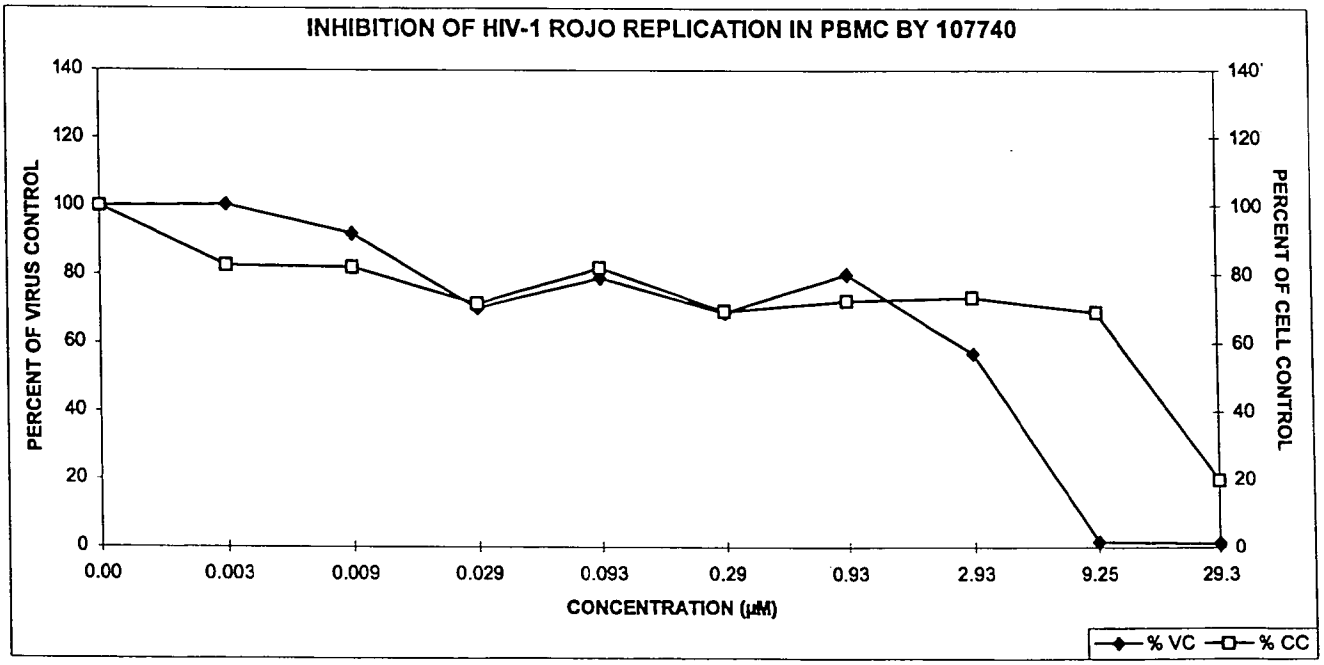
Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 4/25/03, 1.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 ROJO REPLICATION IN PBMC BY 107740

RT Values(cpm)										
CONC (µM)	0.00	0.003	0.009	0.029	0.093	0.29	0.93	2.93	9.25	29.3
SAMPLE 1	41114	61289	70465	60820	80047	35555	34947	9161	699	504
SAMPLE 2	38153	37405	25194	9751	4530	28240	10638	22509	713	497
SAMPLE 3	29862	10966	4650	6220	1807	11450	41909	30556	382	396
MEAN	36376.0	36553.3	33436.3	25597.0	28794.7	25081.7	29164.7	20742.0	598.0	465.7
% VC	100.0	100.5	91.9	70.4	79.2	69.0	80.2	57.0	1.6	1.3

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (µM)	0.00	0.003	0.009	0.029	0.093	0.29	0.93	2.93	9.25	29.3
SAMPLE 1	0.628	0.480	0.495	0.459	0.556	0.483	0.514	0.538	0.482	0.120
SAMPLE 2	0.705	0.623	0.602	0.479	0.556	0.465	0.507	0.479	0.480	0.142
SAMPLE 3	0.703	0.580	0.577	0.521	0.557	0.464	0.454	0.477	0.443	0.142
MEAN	0.679	0.561	0.558	0.486	0.557	0.471	0.492	0.498	0.468	0.134
% CC	100.0	82.7	82.2	71.6	82.0	69.4	72.5	73.4	69.0	19.8

IC50 (µM) =3.39	TC50 (µM) =14.4	TI = 4.25



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

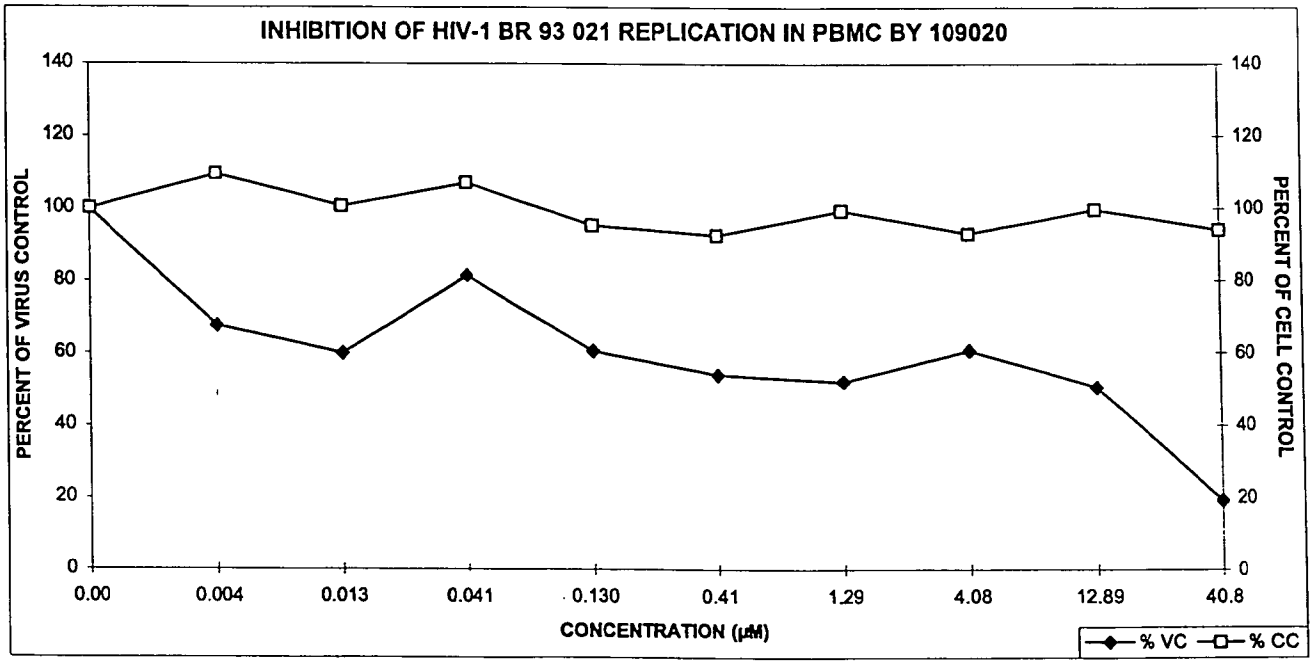
Virus/Strain: HIV-1 / ROJO
Virus Date/Titer: 12/16/02, 3.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 BR 93 021 REPLICATION IN PBMC BY 109020

RT Values(cpm)										
CONC (μM)	0.00	0.004	0.013	0.041	0.130	0.41	1.29	4.08	12.89	40.8
SAMPLE 1	15509	7444	8095	8641	7087	7119	7592	6806	7198	2417
SAMPLE 2	12428	11391	9102	11265	8199	7400	7843	7649	6308	1890
SAMPLE 3	16750	11449	9673	16546	11894	9699	7969	12758	9107	4344
MEAN	14895.3	10094.7	8956.7	12150.7	9060.0	8072.7	7801.3	9071.0	7537.7	2883.7
% VC	100.0	67.8	60.1	81.6	60.8	54.2	52.4	60.9	50.6	19.4

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.004	0.013	0.041	0.130	0.41	1.29	4.08	12.89	40.8
SAMPLE 1	0.983	1.027	0.900	0.981	0.889	0.775	0.907	0.817	0.847	0.813
SAMPLE 2	0.858	0.937	0.928	1.033	0.850	0.900	0.869	0.805	0.810	0.872
SAMPLE 3	0.805	0.932	0.835	0.819	0.784	0.773	0.855	0.838	0.974	0.802
MEAN	0.882	0.966	0.887	0.944	0.841	0.816	0.877	0.820	0.877	0.829
% CC	100.0	109.5	100.6	107.1	95.4	92.5	99.4	93.0	99.5	94.0

IC50 (μM) =13.2	TC50 (μM) =>40.8	TI = >3.09



Client: IR&D
Investigator: Ptak
Setup Date: 04/02/04

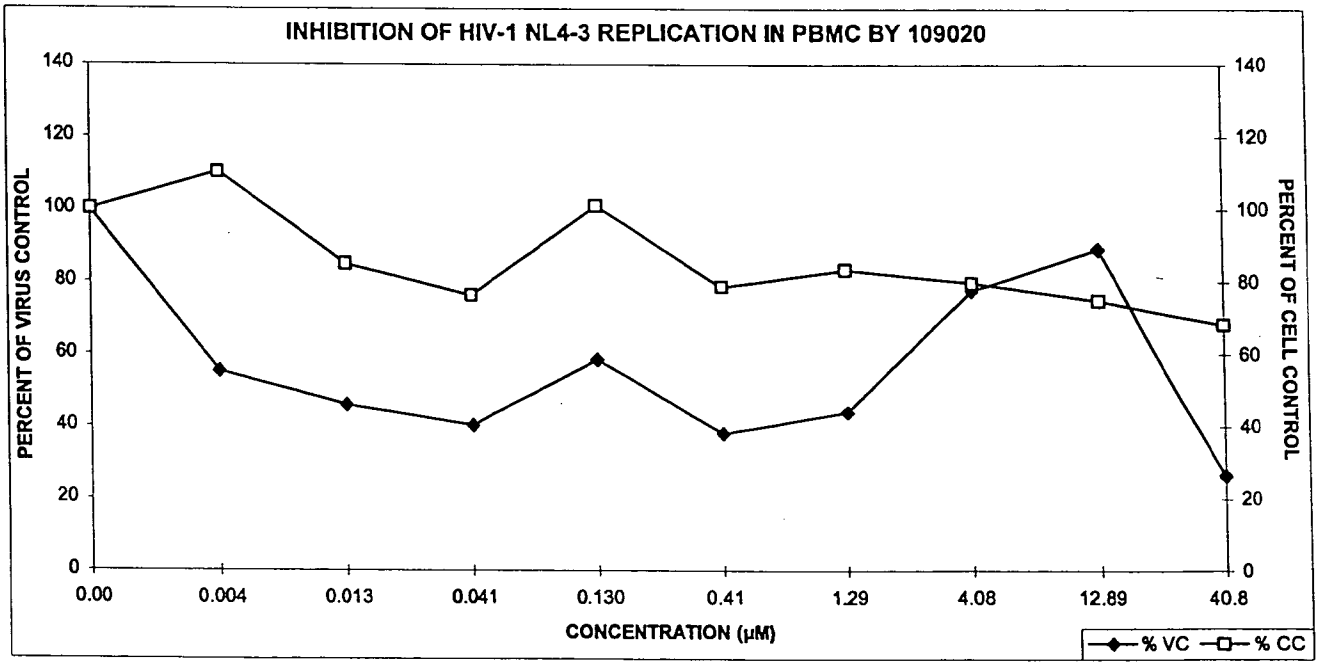
Virus/Strain: HIV-1 / BR 93 021
Virus Date/Titer: 10/18/99, 3.3 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY 109020

RT Values(cpm)										
CONC (µM)	0.00	0.004	0.013	0.041	0.130	0.41	1.29	4.08	12.89	40.8
SAMPLE 1	43455	24885	9221	13637	18067	18740	14614	28060	36905	7195
SAMPLE 2	29494	29215	26514	16139	12141	14577	13912	36908	22372	11681
SAMPLE 3	40549	8636	16515	15997	36245	10024	21491	23193	41739	11065
MEAN	37832.3	20912.0	17416.7	15257.7	22151.0	14447.0	16672.3	29387.0	33672.0	9980.3
% VC	100.0	55.3	46.0	40.3	58.6	38.2	44.1	77.7	89.0	26.4

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (µM)	0.00	0.004	0.013	0.041	0.130	0.41	1.29	4.08	12.89	40.8
SAMPLE 1	0.628	0.809	0.504	0.539	0.789	0.508	0.594	0.513	0.478	0.451
SAMPLE 2	0.705	0.662	0.520	0.486	0.547	0.478	0.470	0.493	0.457	0.451
SAMPLE 3	0.703	0.772	0.703	0.528	0.718	0.616	0.632	0.617	0.589	0.490
MEAN	0.679	0.748	0.576	0.518	0.685	0.534	0.565	0.541	0.508	0.464
% CC	100.0	110.2	84.8	76.3	100.9	78.7	83.3	79.7	74.9	68.4

IC50 (µM) =26.4	TC50 (µM) =>40.8	TI = >1.55



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

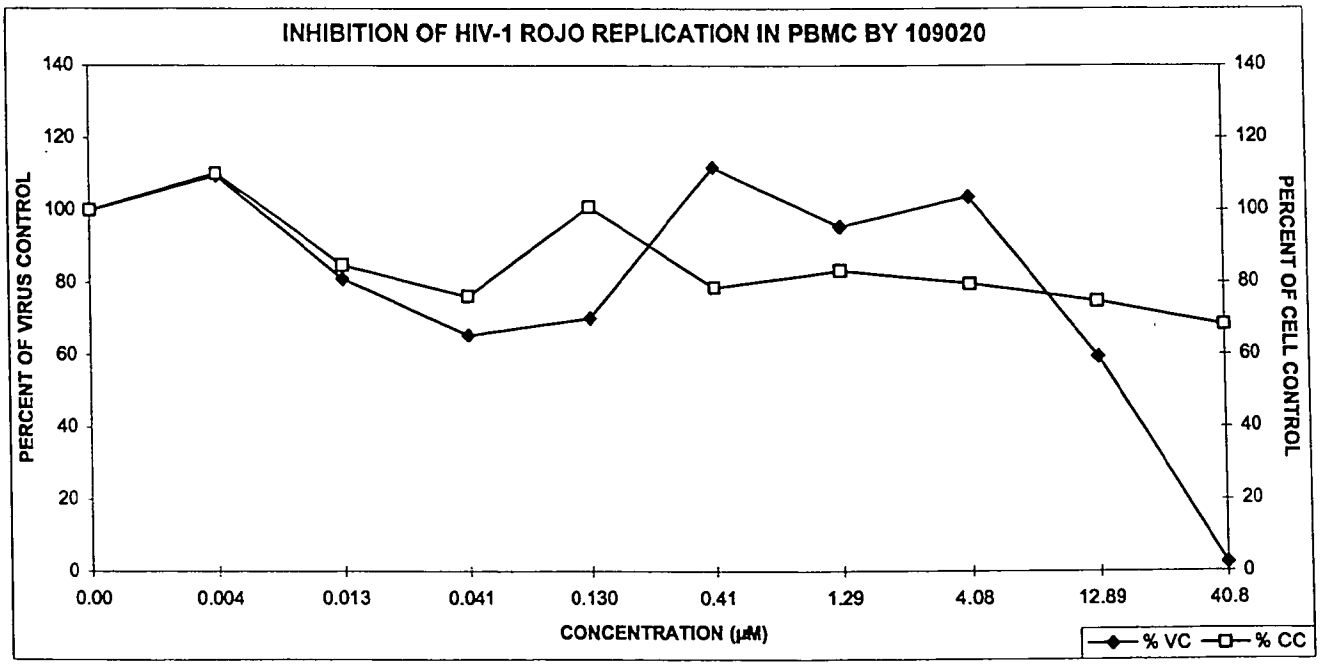
Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 4/25/03, 1.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 ROJO REPLICATION IN PBMC BY 109020

RT Values(cpm)										
CONC (μM)	0.00	0.004	0.013	0.041	0.130	0.41	1.29	4.08	12.89	40.8
SAMPLE 1	41114	72439	11377	39100	45869	48889	33748	13751	19163	1531
SAMPLE 2	38153	27192	33743	13794	16484	20311	6685	55388	4102	491
SAMPLE 3	29862	19969	43511	18680	14266	52876	63653	44004	41713	637
MEAN	36376.0	39866.7	29543.7	23858.0	25539.7	40692.0	34695.3	37714.3	21659.3	886.3
% VC	100.0	109.6	81.2	65.6	70.2	111.9	95.4	103.7	59.5	2.4

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (μM)	0.00	0.004	0.013	0.041	0.130	0.41	1.29	4.08	12.89	40.8
SAMPLE 1	0.628	0.809	0.504	0.539	0.789	0.508	0.594	0.513	0.476	0.451
SAMPLE 2	0.705	0.662	0.520	0.486	0.547	0.478	0.470	0.493	0.457	0.451
SAMPLE 3	0.703	0.772	0.703	0.528	0.718	0.616	0.632	0.617	0.589	0.490
MEAN	0.679	0.748	0.576	0.518	0.685	0.534	0.565	0.541	0.508	0.464
% CC	100.0	110.2	84.8	76.3	100.9	78.7	83.3	79.7	74.9	68.4

IC50 (μM) =15.6	TC50 (μM) =>40.8	TI = >2.62



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

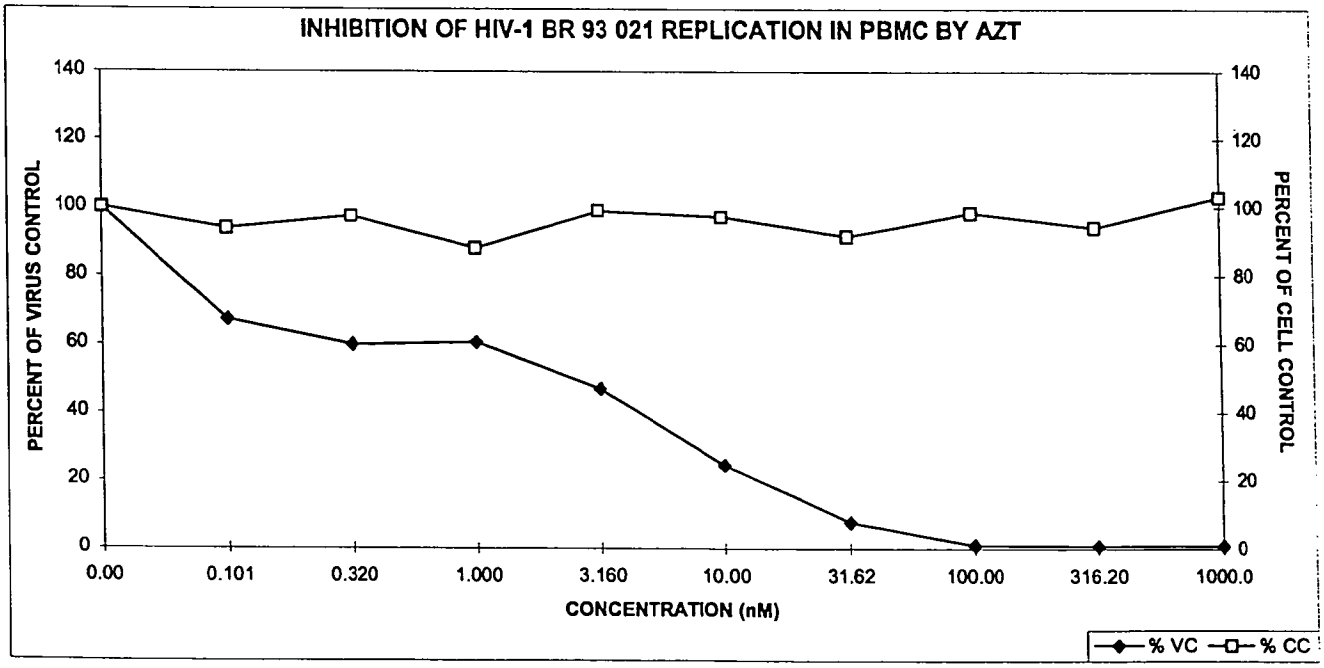
Virus/Strain: HIV-1 / ROJO
Virus Date/Titer: 12/16/02, 3.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 BR 93 021 REPLICATION IN PBMC BY AZT

RT Values(cpm)										
CONC (nM)	0.00	0.101	0.320	1.000	3.160	10.00	31.62	100.00	316.20	1000.0
SAMPLE 1	17550	17694	13145	10124	8718	6375	1602	238	187	115
SAMPLE 2	16422	10558	9582	12190	8074	2978	1288	144	101	231
SAMPLE 3	21459	9146	10528	11325	9318	4281	1376	216	216	144
MEAN	18477.0	12466.0	11085.0	11213.0	8703.3	4544.7	1422.0	199.3	168.0	163.3
% VC	100.0	67.5	60.0	60.7	47.1	24.6	7.7	1.1	0.9	0.9

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (nM)	0.00	0.101	0.320	1.000	3.160	10.00	31.62	100.00	316.20	1000.0
SAMPLE 1	0.834	0.850	0.796	0.779	0.809	0.817	0.749	0.893	0.755	0.837
SAMPLE 2	0.764	0.691	0.882	0.646	0.837	0.747	0.762	0.759	0.767	0.763
SAMPLE 3	0.801	0.716	0.662	0.693	0.732	0.770	0.689	0.710	0.738	0.873
MEAN	0.800	0.752	0.780	0.706	0.792	0.778	0.733	0.787	0.753	0.824
% CC	100.0	94.0	97.5	88.3	99.1	97.3	91.6	98.4	94.2	103.1

IC50 (nM) =2.47	TC50 (nM) =>1000.0	TI = >404.86



Client: IR&D
Investigator: Ptak
Setup Date: 04/02/04

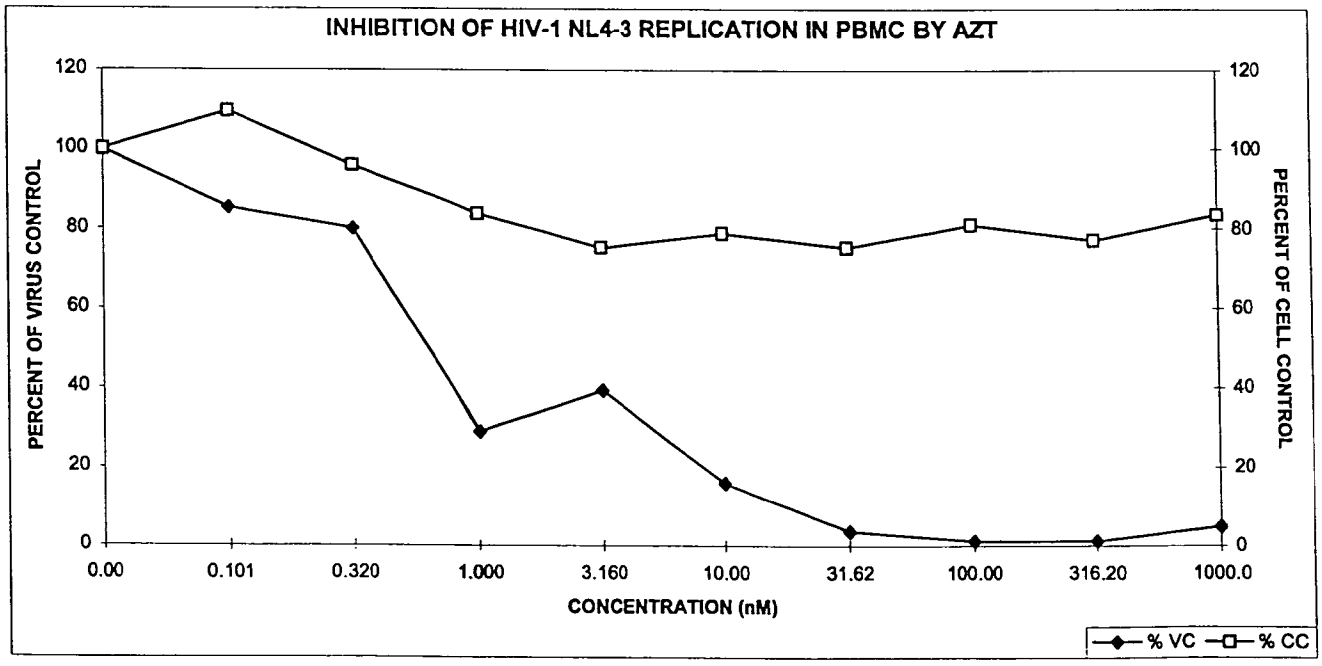
Virus/Strain: HIV-1 / BR 93 021
Virus Date/Titer: 10/18/99, 3.3 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY AZT

RT Values(cpm)										
CONC (nM)	0.00	0.101	0.320	1.000	3.160	10.00	31.62	100.00	316.20	1000.0
SAMPLE 1	38042	28813	31077	17751	23015	4742	717	303	252	310
SAMPLE 2	22726	20019	31795	4369	9084	7993	2590	187	721	4387
SAMPLE 3	42230	38989	19520	7569	8440	3630	461	764	209	346
MEAN	34332.3	29273.7	27464.0	9896.3	13513.0	5455.0	1256.0	418.0	394.0	1681.0
% VC	100.0	85.3	80.0	28.8	39.4	15.9	3.7	1.2	1.1	4.9

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (nM)	0.00	0.101	0.320	1.000	3.160	10.00	31.62	100.00	316.20	1000.0
SAMPLE 1	0.633	0.649	0.631	0.639	0.516	0.580	0.523	0.567	0.617	0.641
SAMPLE 2	0.738	0.830	0.705	0.494	0.518	0.514	0.519	0.493	0.511	0.553
SAMPLE 3	0.702	0.792	0.651	0.605	0.529	0.543	0.521	0.623	0.471	0.539
MEAN	0.691	0.757	0.663	0.579	0.521	0.546	0.521	0.561	0.533	0.578
% CC	100.0	109.5	95.9	83.8	75.3	78.9	75.4	81.2	77.2	83.6

IC50 (nM) =0.62	TC50 (nM) =>1000.0	TI = >1612.90



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

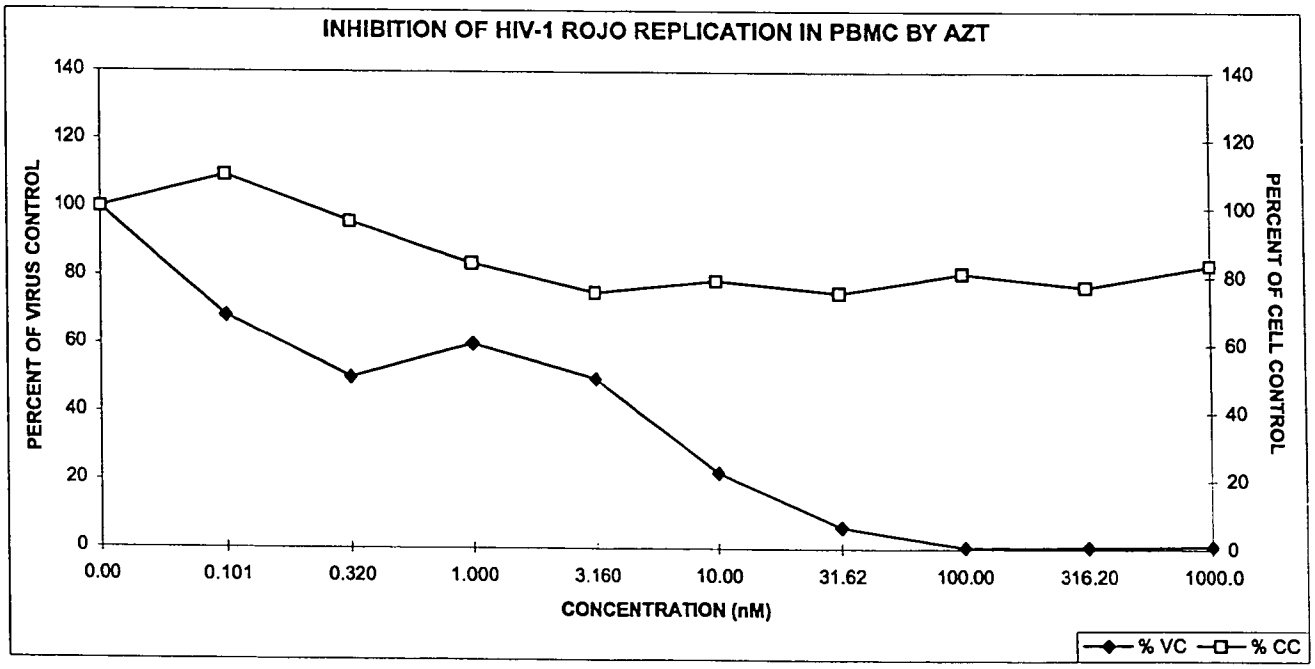
Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 4/25/03, 1.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 ROJO REPLICATION IN PBMC BY AZT

RT Values(cpm)										
CONC (nM)	0.00	0.101	0.320	1.000	3.160	10.00	31.62	100.00	316.20	1000.0
SAMPLE 1	68105	39025	39118	80185	36096	9377	263	418	511	468
SAMPLE 2	38159	53577	22089	1368	15875	2671	263	339	411	555
SAMPLE 3	74099	30952	29627	26983	38250	28136	10798	274	252	468
MEAN	60121.0	41184.7	30278.0	36178.7	30073.7	13394.7	3774.7	343.7	391.3	497.0
% VC	100.0	68.5	50.4	60.2	50.0	22.3	6.3	0.6	0.7	0.8

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (nM)	0.00	0.101	0.320	1.000	3.160	10.00	31.62	100.00	316.20	1000.0
SAMPLE 1	0.633	0.649	0.631	0.639	0.516	0.580	0.523	0.567	0.617	0.641
SAMPLE 2	0.738	0.830	0.705	0.494	0.518	0.514	0.519	0.493	0.511	0.553
SAMPLE 3	0.702	0.792	0.651	0.605	0.529	0.543	0.521	0.623	0.471	0.539
MEAN	0.691	0.757	0.663	0.579	0.521	0.546	0.521	0.561	0.533	0.578
% CC	100.0	109.5	95.9	83.8	75.3	78.9	75.4	81.2	77.2	83.6

IC50 (nM) =3.16	TC50 (nM) =>1000.0	TI = >316.46



Client: IR&D
Investigator: Ptak
Setup Date: 03/19/04

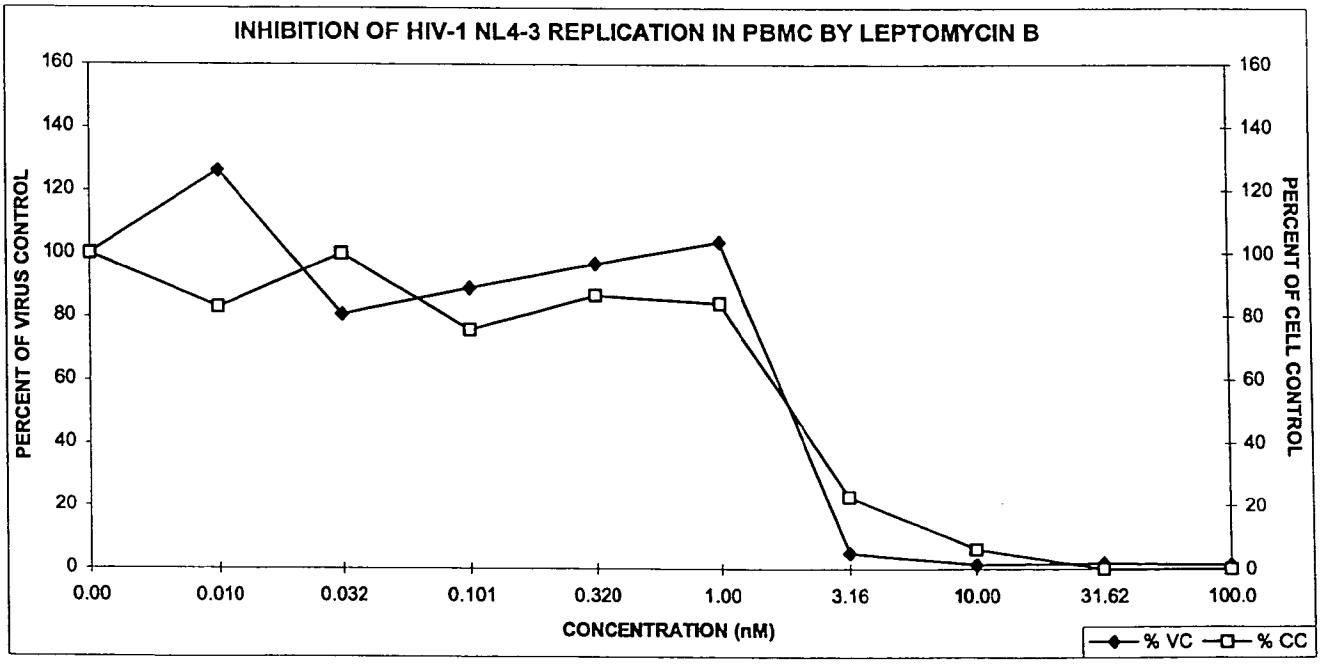
Virus/Strain: HIV-1 / ROJO
Virus Date/Titer: 12/16/02, 3.2 uL/well
Technician: Mankowski

INHIBITION OF HIV-1 NL4-3 REPLICATION IN PBMC BY LEPTOMYCIN B

RT Values(cpm)										
CONC (nM)	0.00	0.010	0.032	0.101	0.320	1.00	3.16	10.00	31.62	100.0
SAMPLE 1	2271	10013	5431	487	4113	9750	561	132	168	88
SAMPLE 2	7854	11856	2796	7341	7289	6417	221	51	59	59
SAMPLE 3	7552	444	6059	7939	5745	2185	103	59	110	59
MEAN	5892.2	7437.7	4762.0	5255.7	5715.7	6117.3	295.0	80.7	112.3	68.7
% VC	100.0	126.2	80.8	89.2	97.0	103.8	5.0	1.4	1.9	1.2

TOXICITY VALUES (Cell Titer 96 - O. D. @ 490/650 nm)										
CONC (nM)	0.00	0.010	0.032	0.101	0.320	1.00	3.16	10.00	31.62	100.0
SAMPLE 1	1.144	0.771	0.782	0.807	0.925	0.900	0.237	0.058	0.000	0.000
SAMPLE 2	0.880	0.795	1.021	0.764	0.825	0.790	0.208	0.065	0.000	0.000
SAMPLE 3	0.911	0.872	1.136	0.657	0.802	0.788	0.226	0.060	0.000	0.000
MEAN	0.978	0.813	0.980	0.743	0.851	0.826	0.223	0.061	0.000	0.000
% CC	100.0	83.1	100.1	75.9	86.9	84.4	22.8	6.2	0.0	0.0

IC50 (nM) =1.87	TC50 (nM) =1.90	TI = 1.02



Client: IR&D
Investigator: Ptak
Setup Date: 03/10/03

Virus/Strain: HIV-1 / NL4-3
Virus Date/Titer: 7/17/02, 0.1 UL
Technician: Mankowski

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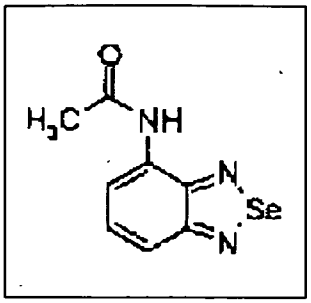
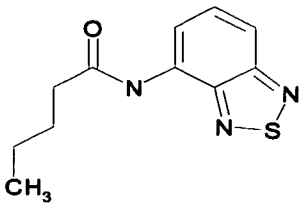
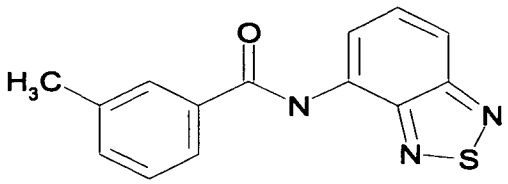
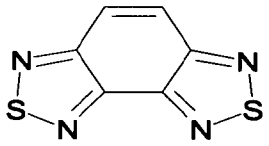
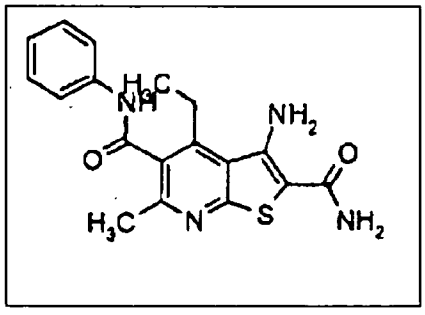
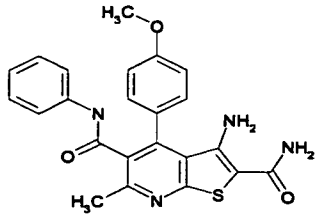
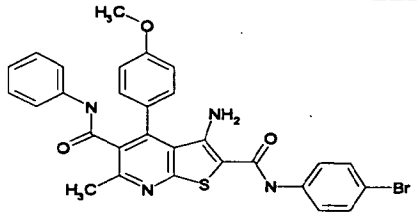
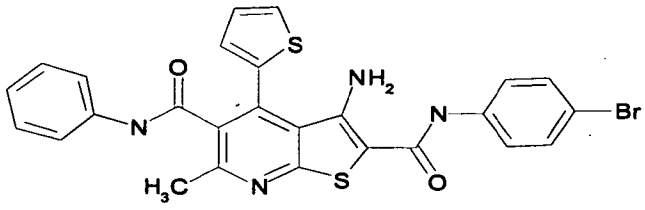
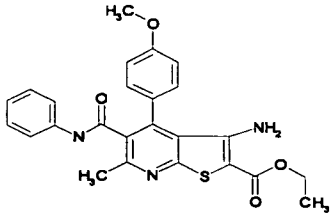
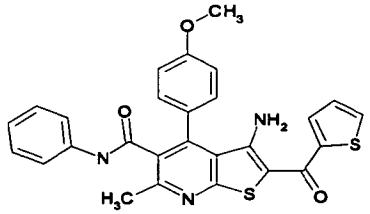
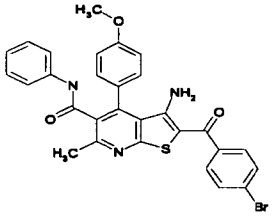
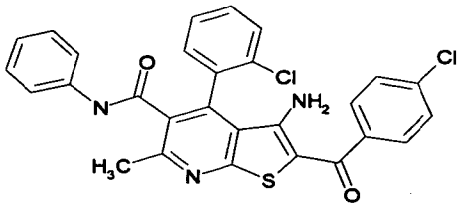
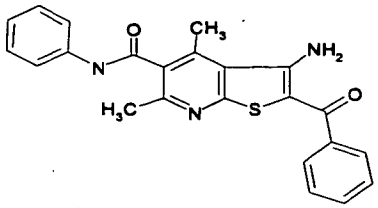
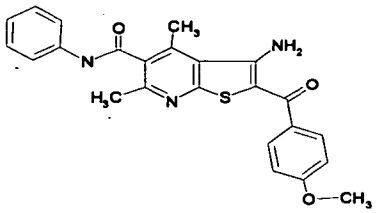
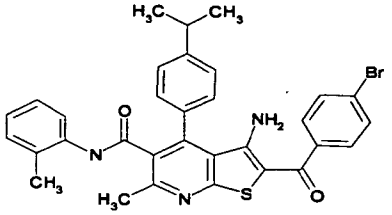
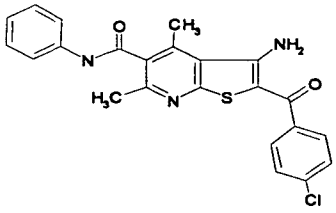
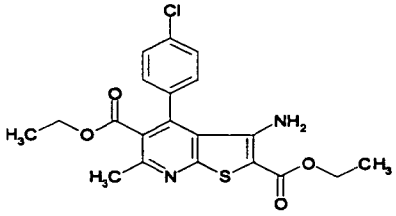
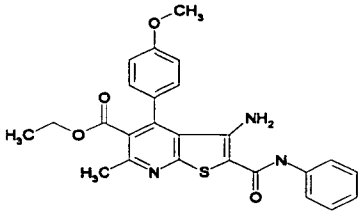
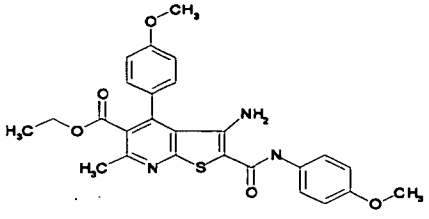
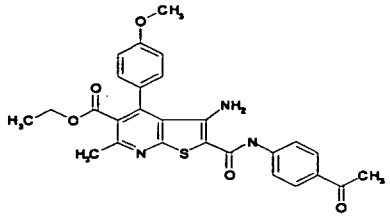
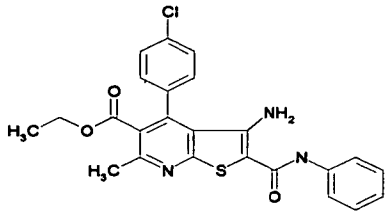
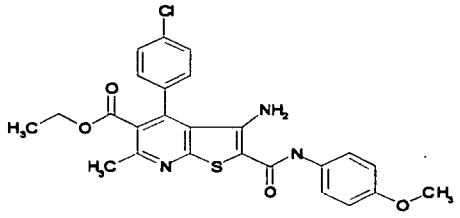
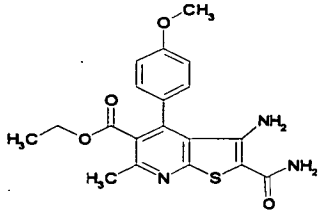
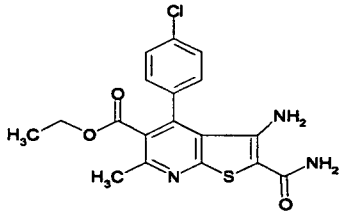
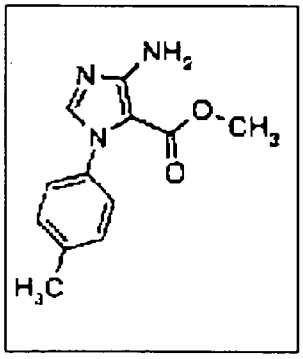
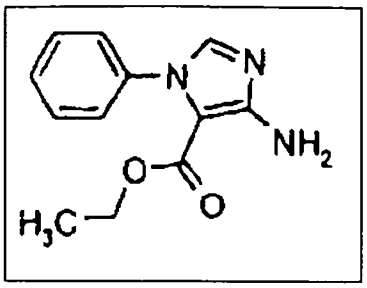
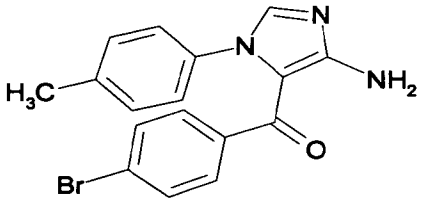
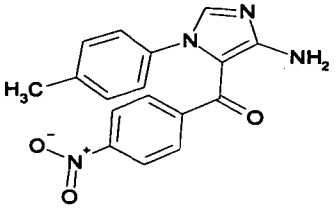
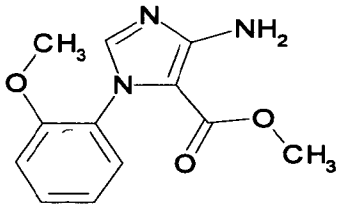
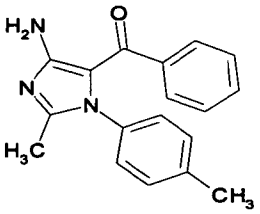
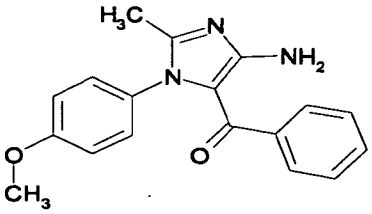
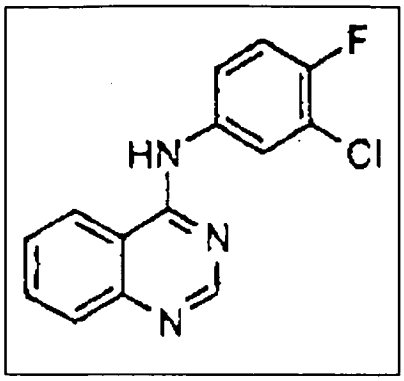
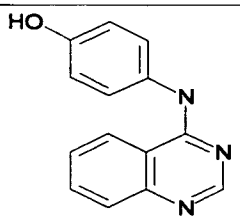
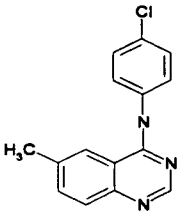
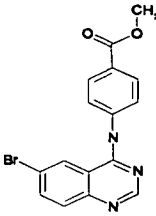
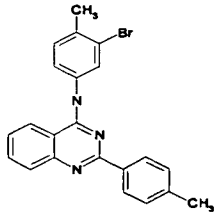
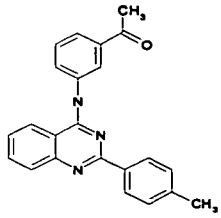
73497	AG-670/31512036	
	AG-690/15441907	
	AG-690/15441921	
	AG-670/31544015	
75168	AE-848/34435026	

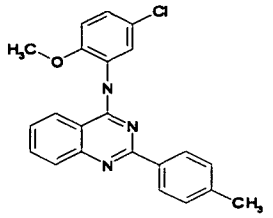
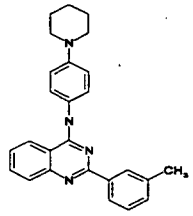
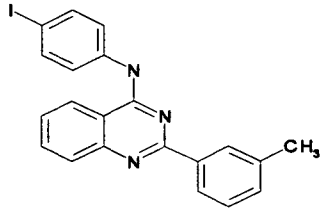
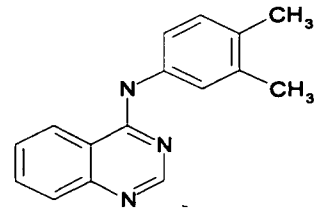
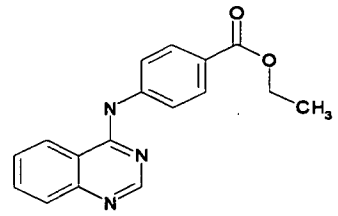
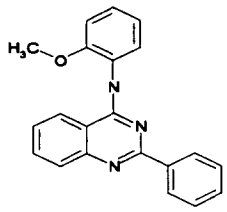
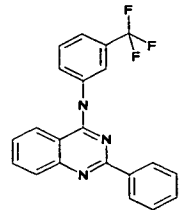
	AE-848/33206051	
	AE-848/33207002	
	AE-848/34089006	
	AE-848/07783052	
	AE-848/08335035	
	AE-848/33766041	
	AE-848/34779006	

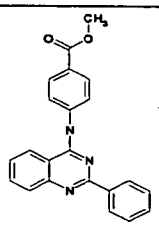
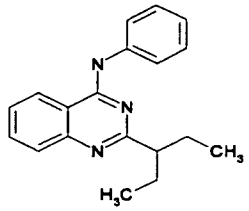
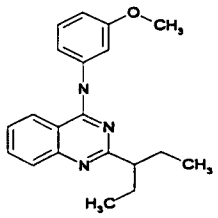
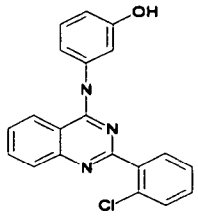
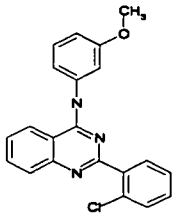
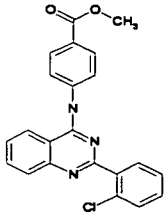
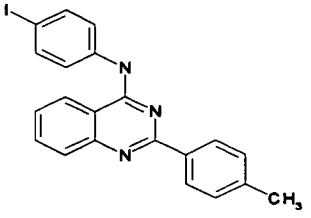
	AG-205/12010037	
	AG-205/12010120	
	AG-205/41004925	
	AG-690/12888651	
	AO-799/42868384	
	AO-799/42007947	
	AO-799/42007949	

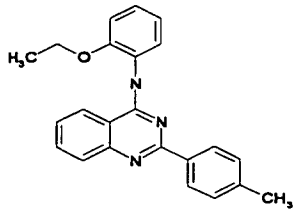
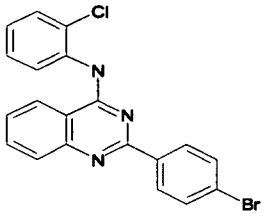
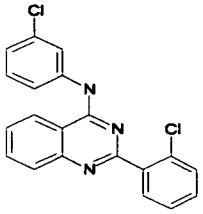
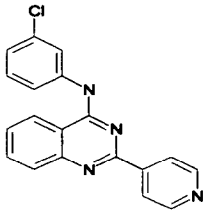
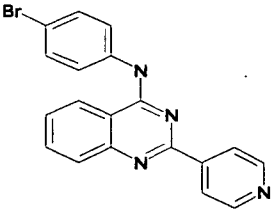
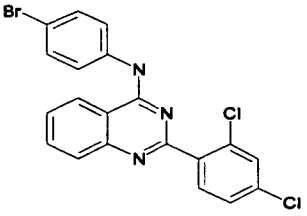
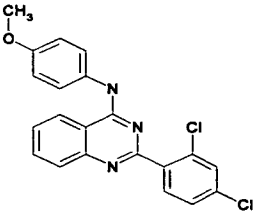
	AO-799/42007950	
	AO-799/42007953	
	AO-799/42007955	
	AO-799/42007991	
	AO-799/42007992	
74377	AH-262/37288007	

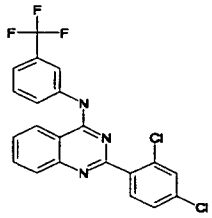
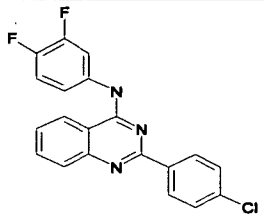
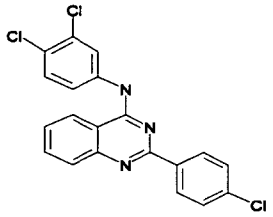
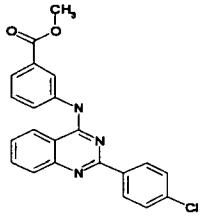
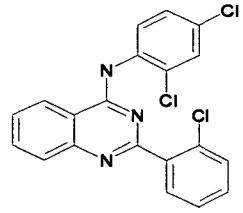
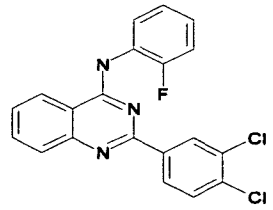
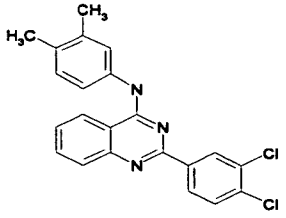
74378	AH-262/37288008	
	AH-262/37093024	
	AH-262/37093030	
	AH-262/37240009	
	AH-262/37295014	
	AH-262/37329001	

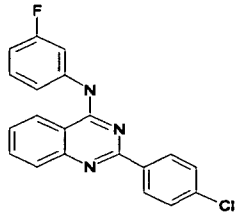
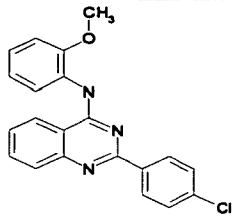
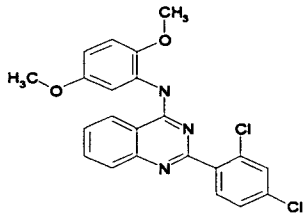
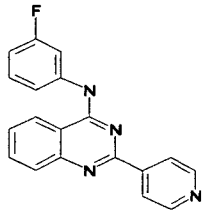
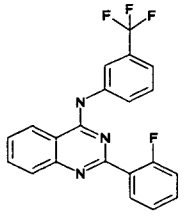
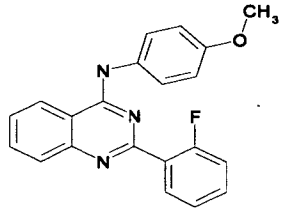
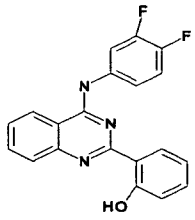
109020	AP-906/41641102	
	AA-504/33316031	
	AE-641/14179112	
	AE-641/14825192	
	AE-641/40975424	
	AE-641/40975489	

	AE-641/40975518	
	AE-641/41040179	
	AE-641/41508835	
	AF-399/13428453	
	AF-399/13428463	
	AF-399/13565053	
	AF-399/13565054	

	AF-399/13567392	
	AF-399/15036519	
	AF-399/15036537	
	AF-399/15284598	
	AF-399/15284599	
	AF-399/15284600	
	AF-399/41147834	

	AF-399/41147837	
	AJ-292/11896050	
	AJ-292/13162008	
	AJ-292/13162009	
	AJ-292/13162013	
	AJ-292/13162014	
	AJ-292/13162017	

	AJ-292/13162020	
	AJ-292/13162021	
	AJ-292/13162022	
	AJ-292/13162025	
	AJ-292/13162051	
	AJ-292/13162052	
	AJ-292/13162055	

	AJ-292/13162057	
	AJ-292/13162059	
	AJ-292/13162060	
	AJ-292/13163134	
	AJ-292/41722570	
	AJ-292/41722653	
	AJ-292/41722682	

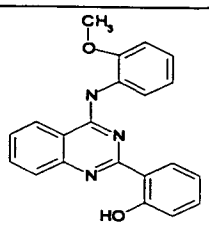
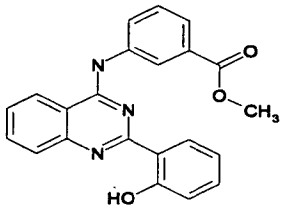
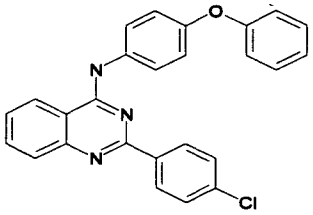
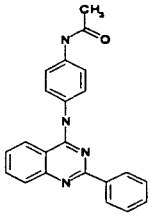
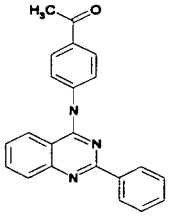
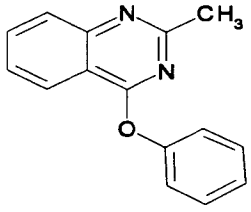
	AJ-292/41722689	
	AJ-292/41722693	
	AP-906/41641069	
	AP-906/41641088	
	AP-906/41641089	
	AE-562/12222957	

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Figure 1A

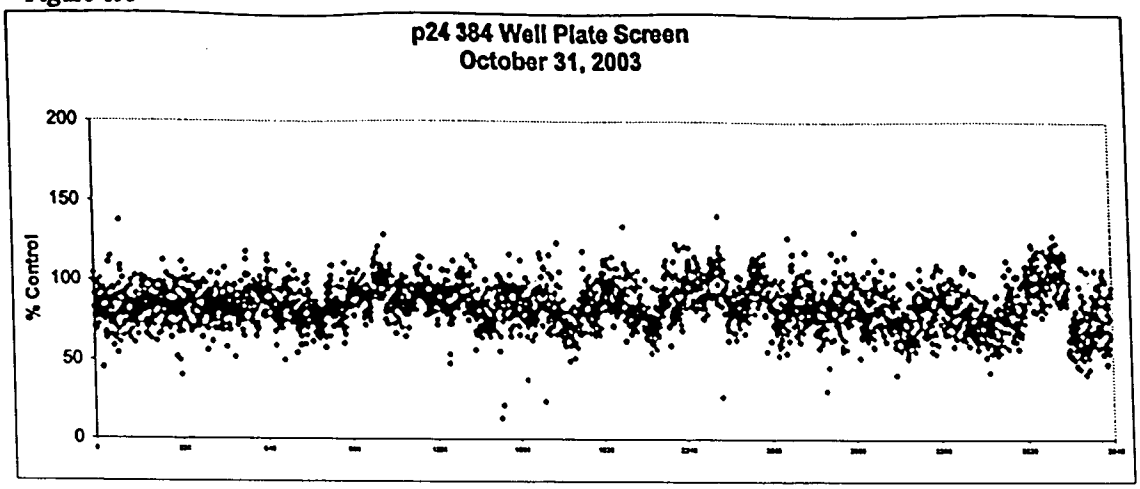


Figure1B

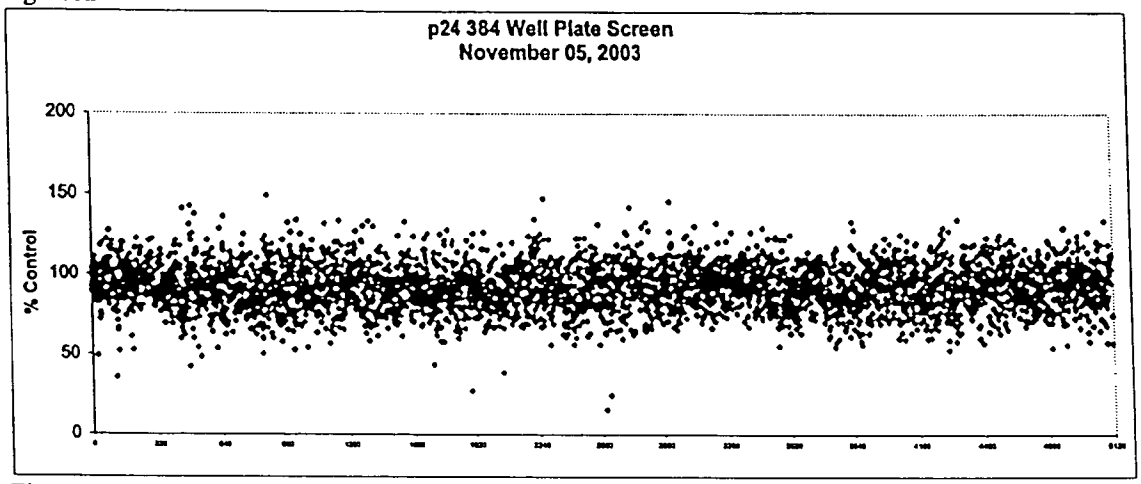
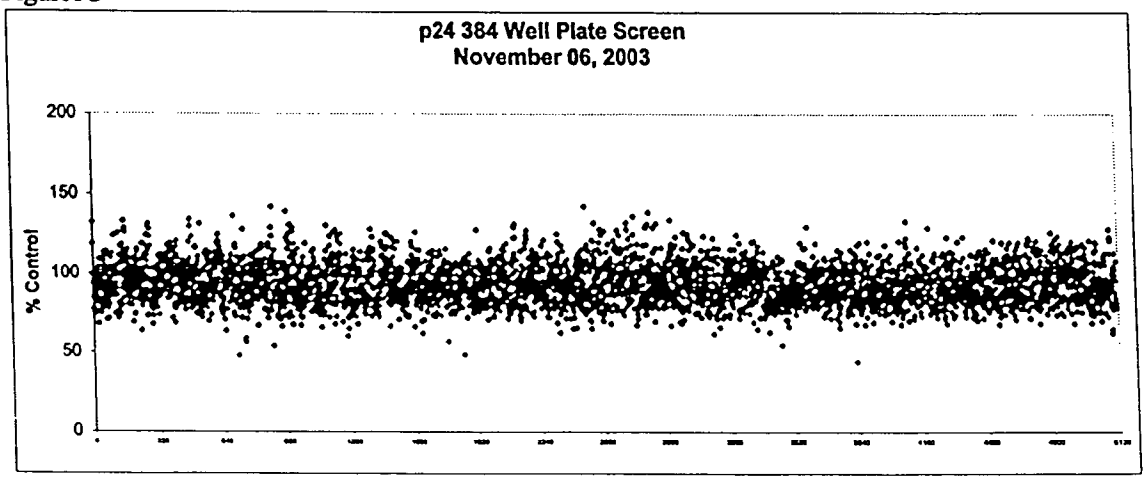


Figure1C



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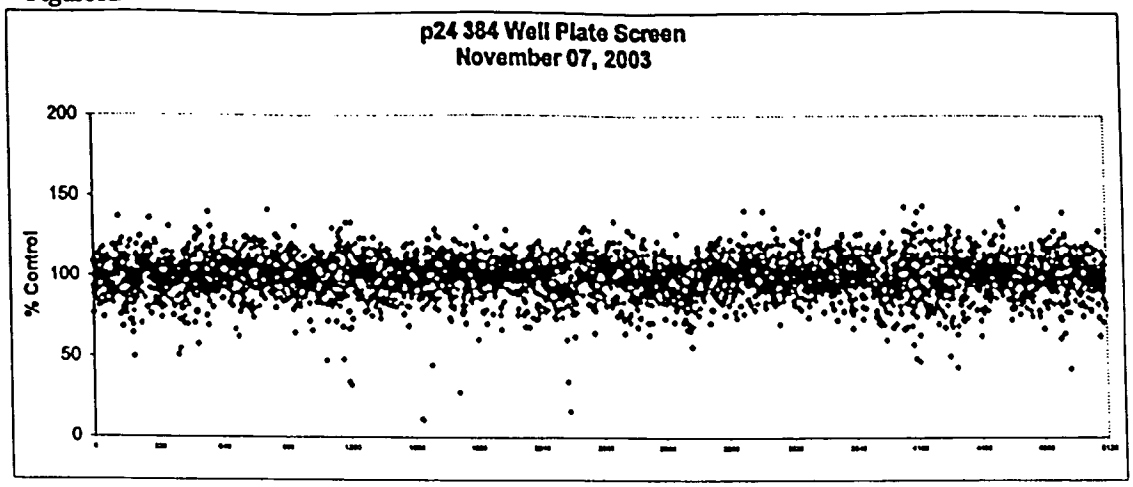

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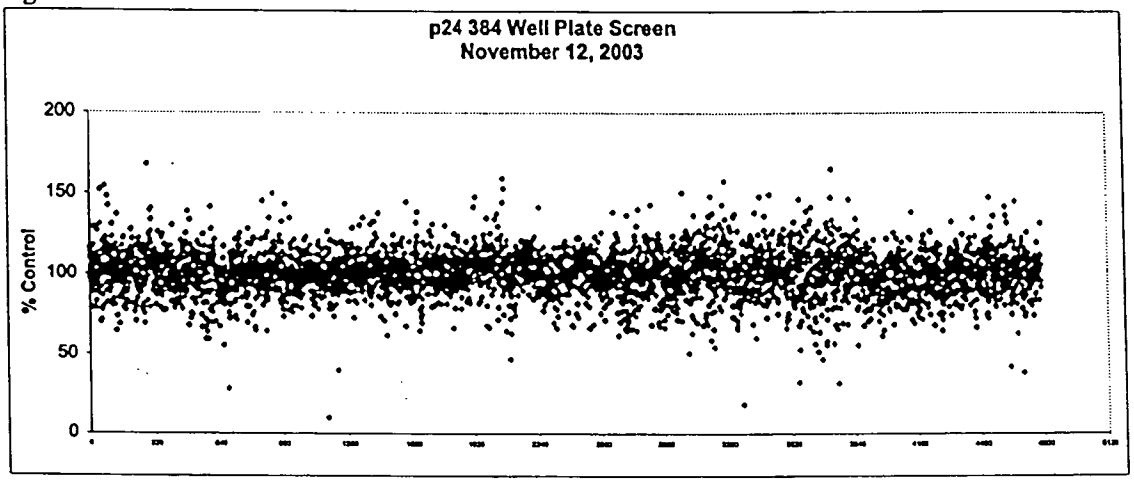


Figure1F

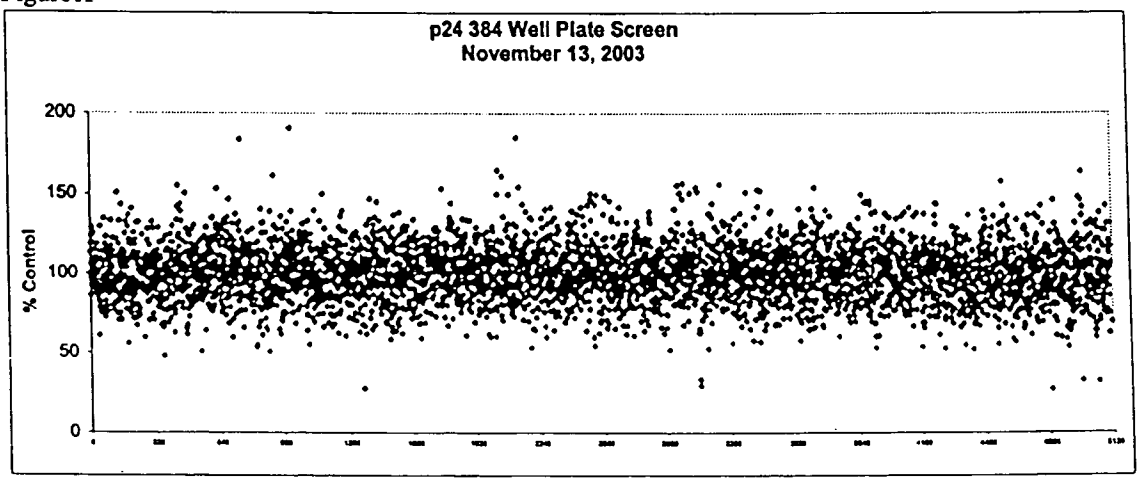
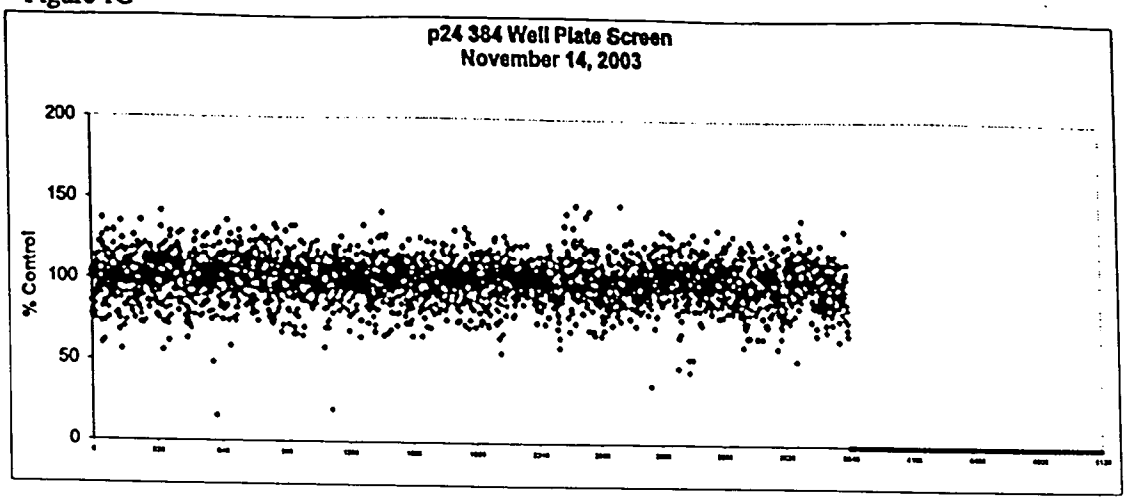


Figure 1G



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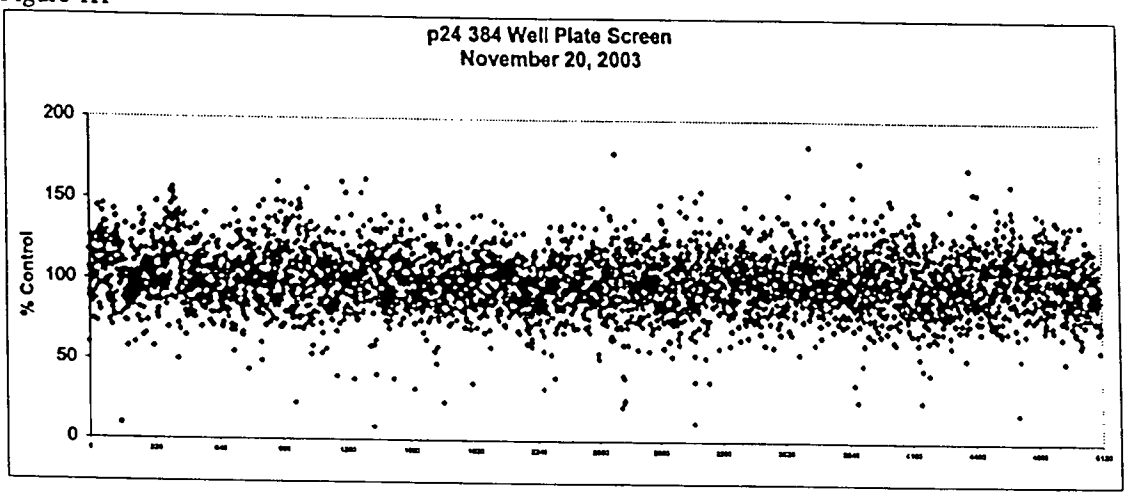

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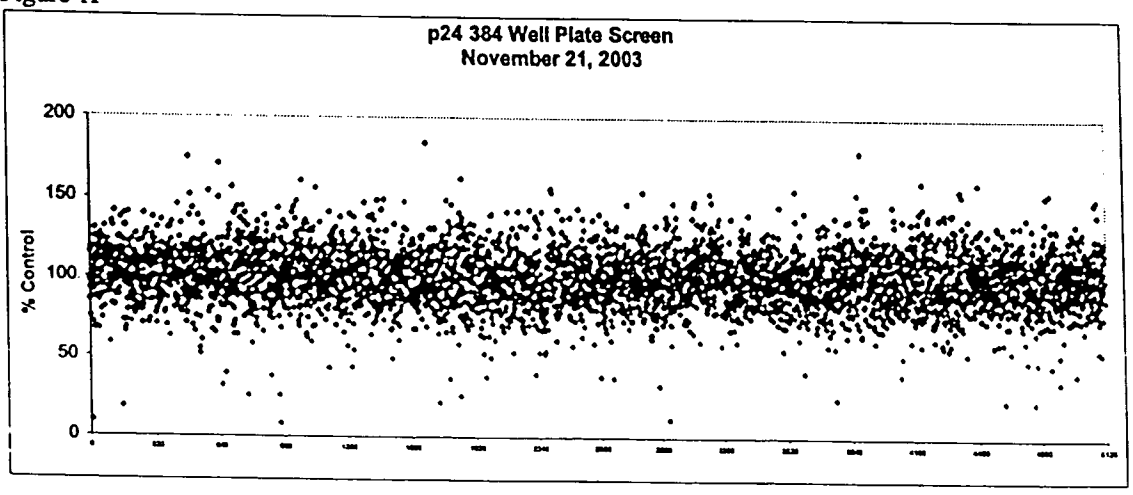

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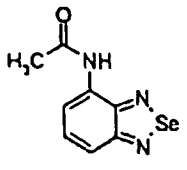
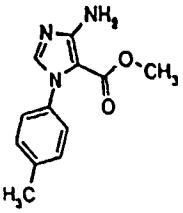
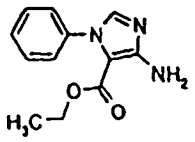
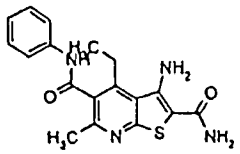
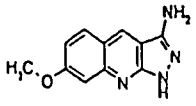
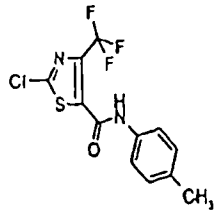
73497 

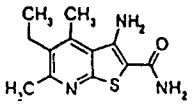
74377 

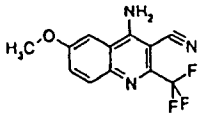
74378 

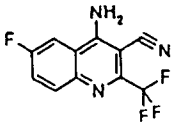
75168 

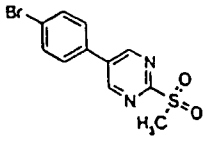
89246 

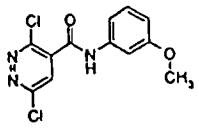
91161 

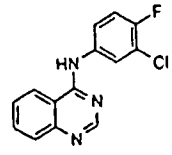
103833 

104366 

106904 

107129 

107740 

109020 

ID-Number	Name
74377	methyl 4-amino-1-(4-methylphenyl)-1H-imidazole-5-carboxylate
74378	ethyl 4-amino-1-phenyl-1H-imidazole-5-carboxylate
73497	N-(2,1,3-benzoselenadiazol-4-yl)acetamide
75168	3-amino-4-ethyl-6-methyl-N~5~-phenylthieno[2,3-b]pyridine-2,5-dicarboxamide
89246	7-methoxy-1H-pyrazolo[3,4-b]quinolin-3-ylamine
91161	2-chloro-N-(4-methylphenyl)-4-(trifluoromethyl)-1,3-thiazole-5-carboxamide
103833	3-amino-5-ethyl-4,6-dimethylthieno[2,3-b]pyridine-2-carboxamide
104366	4-amino-6-methoxy-2-(trifluoromethyl)-3-quinolinecarbonitrile
106904	4-amino-6-fluoro-2-(trifluoromethyl)-3-quinolinecarbonitrile
107129	5-(4-aminophenyl)-2-pyrimidinethiol
107740	3,6-dichloro-N-(3-methoxyphenyl)-4-pyridazinecarboxamide
109020	N-(3-chloro-4-fluorophenyl)-N-(4-quinazolinyl)amine

Fig. 3: 3-Point Dose Response of P24

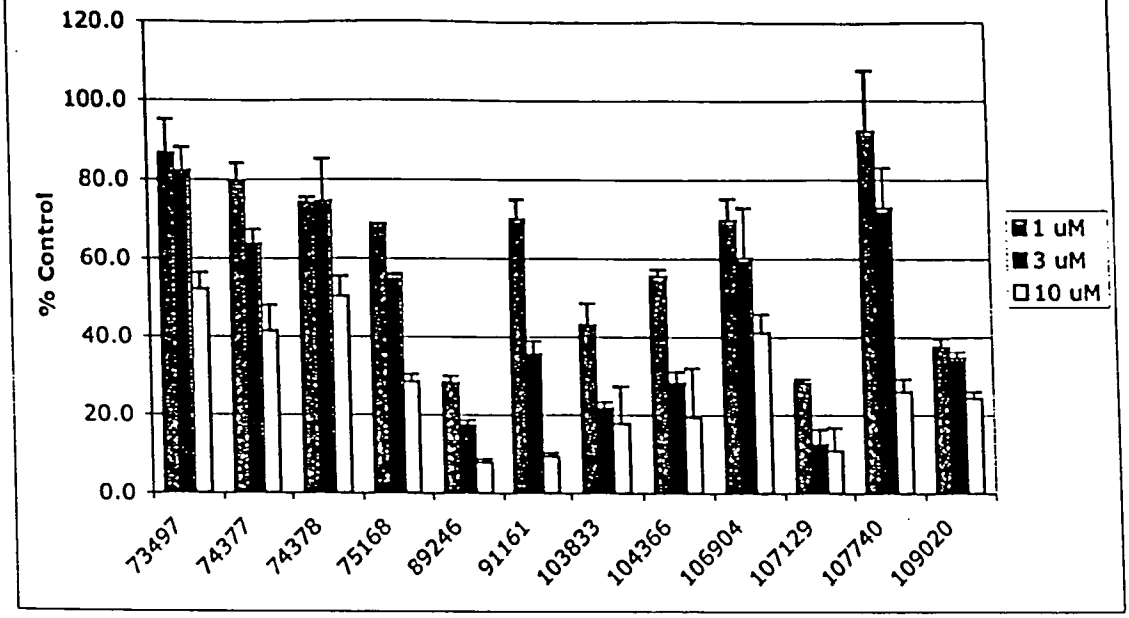
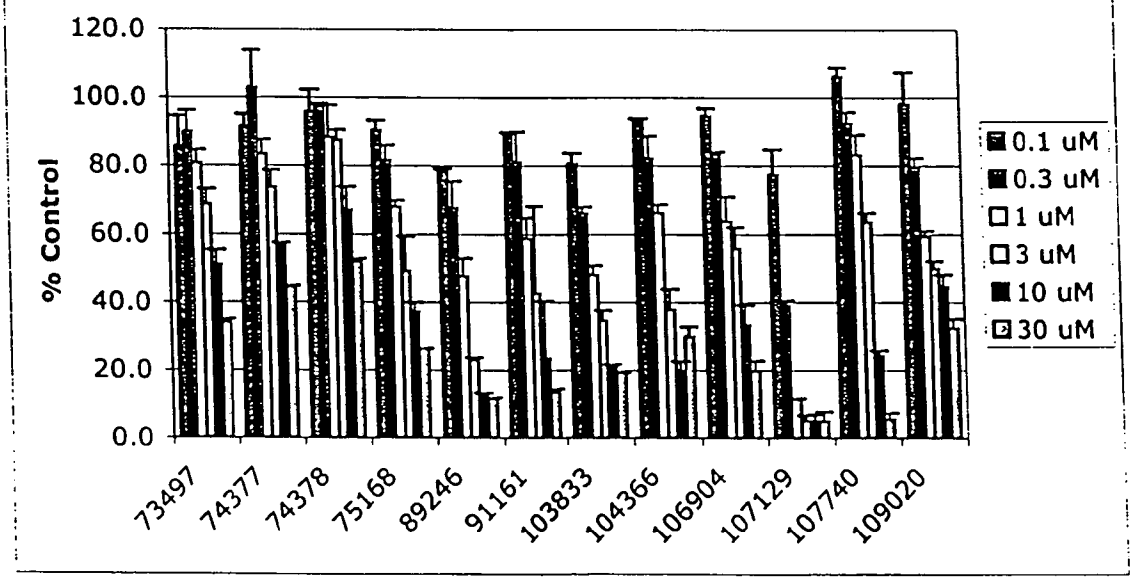


Fig. 4: 6-Point Dose Response of P24



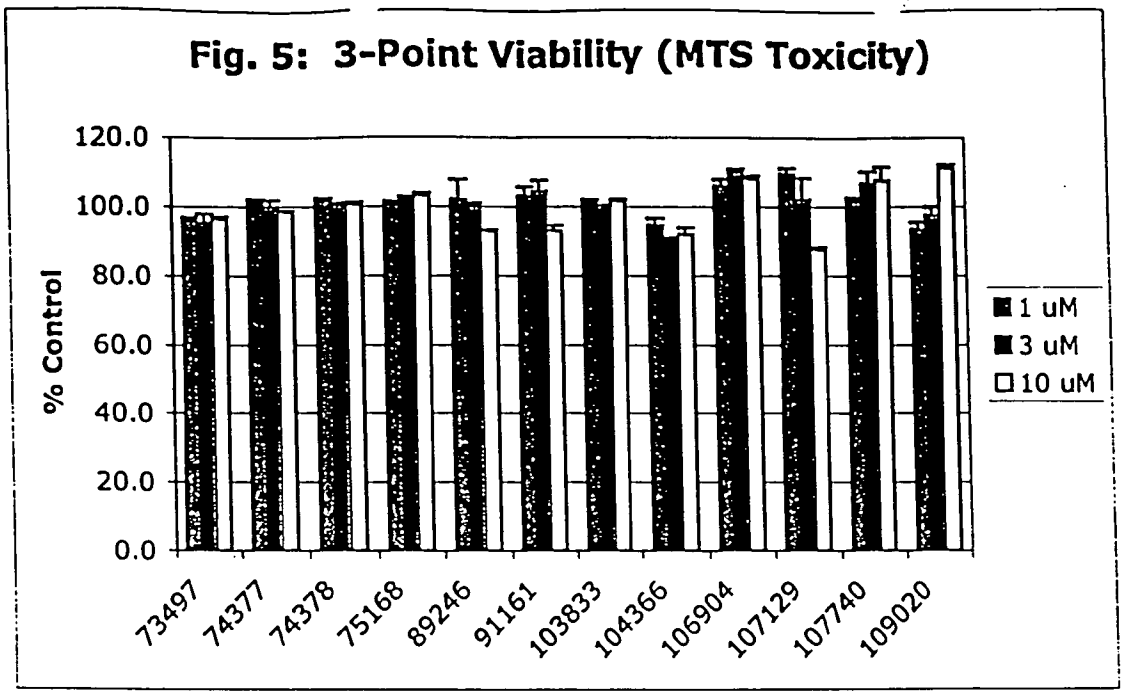


Fig. 5: 3-Point Viability (MTS Toxicity)
% Control
120.0
100.0
80.0
60.0
40.0
20.0
0.0
73497
74377
74378
75168
89246
91161
103833
104366
106904
107129
107740
109020
1 uM
3 uM
10 uM

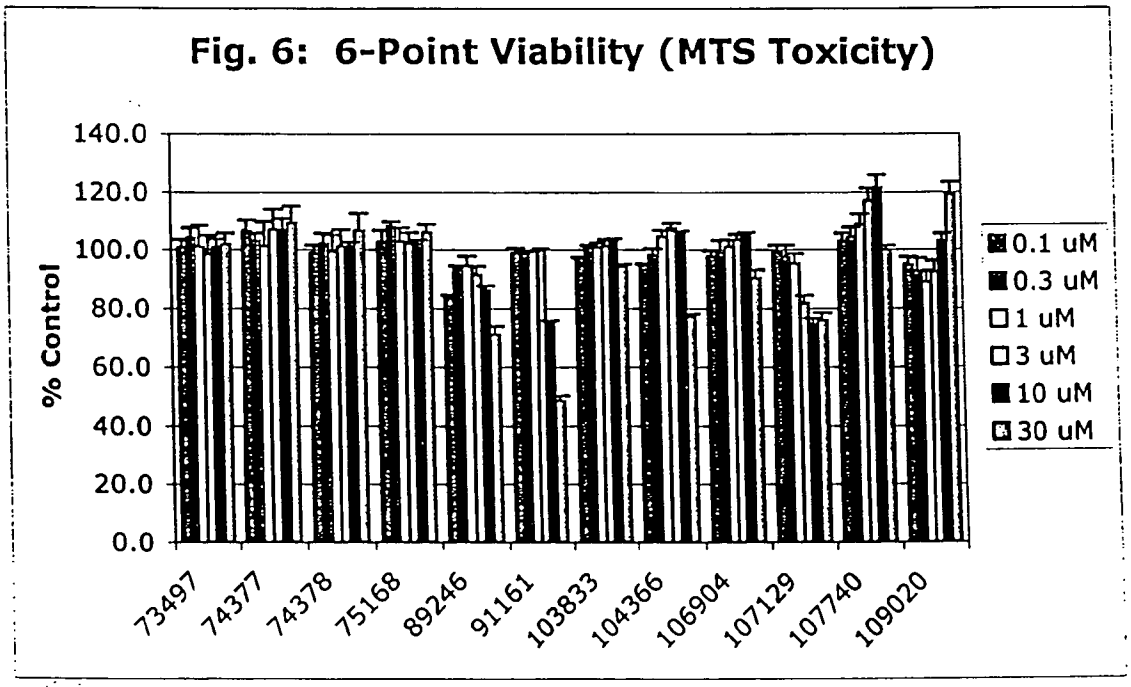


Fig. 6: 6-Point Viability (MTS Toxicity)
% Control
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
73497
74377
74378
75168
89246
91161
103833
104366
106904
107129
107740
109020
0.1 uM
0.3 uM
1 uM
3 uM
10 uM
30 uM

Fig. 7: 6-Point MT4 Viability